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REVIEW ARTICLE

Clinical significance of breast cancer micrometastasis in the sentinel lymph node

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Abstract The advantages of sentinel lymph node biopsy (SLNB) in breast cancer patients include an enhanced pathological examination of a small number of sentinel lymph nodes (SLNs), which permits more frequent detection of micrometastasis and isolated tumor cells (ITCs). At the same time, however, SLNB raises two new concerns: whether minimal SLN involvement has a significant impact on survival and whether patients with such minimal involvement should undergo further axillary dissections. Two large randomized studies, ACOSOG Z0011 and IBCSG 23-01, have demonstrated that axillary lymph node dissection (ALND) can be avoided for select SLN-positive patients. However, for patients with macrometastasis in SLN or who do not meet the inclusion criteria of the two studies, ALND is still the standard management. On the other hand, previous studies appear to disagree on the prognostic significance of minimal SLN involvement. One of the reasons for this discrepancy is the great variability among pathological examinations for SLN. The OSNA method, which is a fast molecular detection procedure targeting cytokeratin 19 (CK19) mRNA, has the advantage of reproducibility among institutions and the capability to examine a whole lymph node within 30–40 min. This novel method may thus be able to overcome the issue of variability among conventional pathological examinations.

Keywords Breast cancer · Sentinel lymph node · Micrometastasis · OSNA

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Introduction

Two decades have passed since sentinel lymph node biopsy (SLNB) was first adopted as a treatment for breast cancer. During the first decade, the feasibility and accuracy of sentinel lymph node (SLN) examination for the prediction of axillary nodal status was confirmed and subsequently the methodology was standardized in numerous studies [1–8]. During the next decade, the survival of SLN-negative patients who underwent no further axillary lymph node dissection (ALND) was investigated. Several studies demonstrated that there was no difference in survival among those who did or did not undergo ALND. This clearly established that SLNB alone was a safe and acceptable procedure for SLN-negative patients [9–11].

The advantages of SLNB are as follows: (1) when the SLNs are negative, ALND can be safely avoided, resulting in less morbidity than with ALND and (2) the enhanced pathological examination of a small number (one or a few) of SLNs permits more frequent detection of micrometastasis and ITC. However, SLNB raises new issues; specifically, whether such small metastases have a measurable impact on survival and whether patients with such small metastases should undergo further axillary dissections. This article will address the issue of the prognostic significance of micrometastasis and ITC and the necessity of ALND for patients with such metastatic disease.

What is the impact of micrometastasis in SLN on survival?

Whether minimal SLN metastases have an impact on survival remains controversial even though many small-scale retrospective studies have attempted to address this issue.

Table 1 Summary of studies assessing prognostic outcome for minimal SLN metastases

Authors	Study design	Year	No. of patients				Length of F/U (mo)	Conclusions
			Total	PN0	PN0 (i+)	PN1mi		
Hansen [12]	Prospective	1992–1999	790	486	84	54	72.5	Pts. with pN0(+i) or pN1mi did not have a worse 8-year DFS or OS than pN0 pts.
Gobardhan [13]	Prospective	2000–2003	1411	922		103	40	DFS and OS were comparable for pN0 and pN1mi pts.
Maaskant-Braat [14]	Population-based	1996–2006	6803	4562	126	451	50	Pts.with pN1mi or pN0(+i) did not have a worse DFS or OS than pN0 pts.
de Boer [15]	Population-based	2006	2707	856	819	1032	61	OS, DFS were worse for pN1mi. than for pN0 pts.
Andersson [16]	Prospective	2000–2004	3369	2383	107	123	52	DFS was worse for pN1mi pts. than for pN0 pts.
Weaver [17]	Prospective	1999–2004	3887	431	186		95.6	OS, DFS, DDFS were worse for occult metastases pts. than for pN0 pts.

SLN sentinel lymph node, *pts* patients, *pN0* tumor-free lymph nodes, *pN0 (i+)* isolated tumor cells, *pN1mi* micrometastases, *F/U* follow-up, *DFS* disease-free survival, *OS* overall survival, *DDFS* distant disease-free survival

Some studies have reported observing a significant impact of micrometastasis and ITC on survival, whereas others have not. Hansen et al. [12] reported finding no differences in disease-free survival (DFS) and overall survival (OS) between patients with micrometastasis and ITC and those without metastatic nodal disease in a prospective analysis of 790 patients with a median follow-up of 72.5 months (Table 1). Gobardhan et al. [13] also reported that DFS and OS were comparable for patients with and without micrometastasis after an adjustment for possible confounding characteristics and for adjuvant systemic treatment. They concluded that survival is not affected by the presence of micrometastasis and ITC in SLN. Similarly, Maaskant-Braat et al. [14]. found no significant difference in overall survival between patients with and without micrometastatic disease, and their results did not change even after adjusting for adjuvant systemic therapy. In sharp contrast to these three studies, the retrospective cohort Micrometastasis and Isolated tumor cells: relevant and robust or rubbish (MIRROR) study found that patients with both micrometastasis and ITC were characterized by a poorer prognosis than those without metastatic nodal disease [15]. This study also demonstrated that adjuvant therapy significantly improved DFS for patients with micrometastasis and ITC. However, the MIRROR trial included patients with favorable tumor characteristics who did not receive adjuvant systemic therapy in accordance with the Dutch guideline at that time. However, the rate of micrometastasis in the MIRROR study was much less than can be expected in daily clinical practice. In addition, the MIRROR trial used DFS as the endpoint. Because the patients with favorable characteristics had a good prognosis, the risk of distant failure was quite low, so that the influence of locoregional recurrence rather than distant recurrence on DFS was relatively high. Another prospective study conducted in Sweden of 3369 patients with a median follow-up of 52 months found that 5-year cause-specific and event-free survival rate was lower for patients with micrometastasis than for node-negative patients. On the other hand, there was no significant difference in survival between node-negative patients and those with isolated tumor cells [16]. Weaver et al. demonstrated in their prospective, multicenter analysis that occult metastases (11.1 % for isolated tumor cells, 4.4 % for micrometastases, 0.4 % for macrometastases) in initially negative SLNs have a small, but significant impact on OS, DFS and distant disease-free interval [17].

Because these studies show substantial discrepancies regarding the prognostic significance of minimal SLN involvement, no definitive conclusions can be drawn. At present, breast cancer patients are treated on the basis of the intrinsic subtype since breast cancers feature quite different prognoses and sensitivity to systemic chemo- and hormonal therapy depending on the intrinsic subtype. To date, however, no studies have been conducted using a subpopulation analysis according to the intrinsic subtypes, even though the impact on survival according to the intrinsic subtypes warrants investigation. Moreover, the great majority of patients enrolled in the above-mentioned studies were estrogen receptor-positive patients whose recurrence often occurs as late as between 5 and 10 years after surgery [18]. However, the mean follow-up periods are usually only approximately 5–6 years, thus longer follow-up

Table 2 Summary of studies assessing the rate of locoregional recurrence of the patients with SLN micrometastases not undergoing ALND

Authors	Study design	Year	No. of patients		Length of F/U (months)	Conclusions
			SLNB alone	SLNB + ALND		
Bilimoria [19]	Population-based	1998–2005	3674	6585	63	Rates of ipsilateral regional events were comparable for pts with and without ALND
Yi [20]	Population-based	1998–2004	2240	4598	50	Rates of ipsilateral regional events were comparable for pts with and without ALND
Pepels [21]	Population-based	1997–2005	141	887	61	5-year regional recurrence was significantly higher for pts not undergoing ALND than those undergoing ALND
Giuliano[a] [22]	Prospective	1999–2004	436	420	52	ALND had no significant impact on locoregional recurrence in pts with SLN metastasis
Galimberti [23]	Prospective	2001–2010	464	465	95.6	ALND had no significant impact on locoregional recurrence in pts with SLN micrometastasis

SLN sentinel lymph node, *ALND* axillary lymph node dissection, *pts* patients, *F/U* follow-up, *DFS* disease-free survival, *OS* overall survival

[a] 41.2 % of patients had micrometastases in SLN

studies are required to determine the impact on survival of minimal SLN involvement.

Is ALND necessary for patients with micrometastasis in SLN?

Whether patients with micrometastasis in SLN require further ALND remains controversial. Two very large population studies have addressed this issue. Bilimoria et al. [19] reported the results of their analysis of approximately 100,000 node-positive patients with a median follow-up of 63 months listed in the US National Cancer Data Base (Table 2). The authors found no significant difference in axillary recurrence between those who underwent SLNB alone and those who underwent ALND for micrometastasis in SLN. Consistent with the finding of this analysis, according to data for 6838 patients with microscopic SLN metastases obtained from the Surveillance Epidemiology and End Results (SEER) database, there were no significant differences in ipsilateral regional recurrence for SLNB alone ($n = 2240$) versus SLNB with completion ALND ($n = 4598$) [20]. However, in the study by Bilimoria et al., patients with macroscopic SLN metastases showed a tendency to have a lower risk of axillary recurrence for SLNB with ALND compared with SLNB alone. Moreover, in the aforementioned study of the SEER database, patients with macrometastases in SLN had a significantly lower risk of developing ipsilateral regional recurrence after ALND than after SLNB alone. These two studies, although acknowledging certain biases, reflect the experience of daily clinical practice. On the other hand, the Dutch MIRROR cohort study demonstrated that not performing ALND for patients with micrometastases was associated with a higher 5-year regional recurrence and showed that doubling of tumor size, grade 3 and negative hormone receptor status were significantly associated with recurrence [21].

Two large randomized studies were conducted to address the impact of ALND on axillary recurrence in SLN-positive patients. In the prospective multicentric American College of Surgeons Oncology Group (ACOSOG) Z0011 trial, approximately 900 patients with T1-2, N0 breast cancer who underwent breast-conserving surgery (BCS) and SLNB with routine hematoxylin- and eosin-detected metastasis in two or less SLNs were randomized to ALND or no further axillary surgery [22]. All patients received tangential whole breast irradiation. After a median follow-up of 6.3 years, no significant differences in OS and DFS were found between the two groups. It was noted that only 4 (0.9 %) of the 446 SLN-positive patients without ALND showed axillary lymph node recurrence. This result showed no significant differences in a comparison with the 2 (0.5 %) out of 445 SLN-positive patients receiving ALND. In another randomized study, the International Breast Cancer Study Group (IBCSG) 23-01, patients with one or more micrometastatic SLNs were randomly assigned to either ALND or no ALND [23]. At a median follow-up of 5.0 years, no significant differences in DFS or OS were found between the two groups. Since all patients in the ACOSOG Z0011 trial and approximately 90 % of the patients in the IBCSG 23-01 trial received adjuvant radiotherapy, the local effect of whole breast irradiation on

the axillary node basin could not be completely excluded. Moreover, 96 % of the patients in the ACOSOG Z0011 trial and 96 % of those in the IBCSG 23-01 trial were treated with chemotherapy and/or hormonal therapy and such treatment may have also eliminated the minimal SLN involvement. Even after routine ALND, the incidence of local failure including axillary recurrence after a lengthy follow-up is reportedly as high as 2.1 % after ALND, which is similar to that for the ACOSOG Z0011 and IBCSG 23-01 trials. In addition, ALND has been associated with a considerable risk of paresthesia, lymphedema, seroma, sensory change and limitation of shoulder motion [24]. The overall findings reported thus far indicate that ALND can be avoided for T1-2 N0 breast cancer patients with one or two micrometastases and ITC who have been treated with BCS and whole breast irradiation and have received adjuvant chemo- and/or hormonal therapy.

For patients with macrometastasis in SLN, however, only one study (ACOSOG Z0011) has investigated the impact of ALND on axillary recurrence. In addition, none of the patients in the ACOSOG Z0011 trial and approximately 9 % of those in the IBCSG 23-01 trial underwent mastectomy, therefore, it can hardly be said that there is sufficient evidence to avoid ALND for patients with macrometastasis in SLN or for those who have undergone mastectomy. Moreover, we believe that the information on the number of positive nodes obtained by means of ALND remains very important to decide whether there is indication for adjuvant chemotherapy and radiation therapy for the chest wall, supraclavicular fossa and internal mammary chain of patients undergoing both BCS and mastectomy [25, 26].

Pathological examination vs. OSNA assay

As demonstrated above, current opinions diverge regarding the impact of minimal SLN metastases on survival. One of the reasons for the discrepancy is the great variability among pathological examinations for SLN. The frequency of micrometastasis and ITC varies widely among studies of SLNB for patients with breast cancer. This is mainly because removed SLNs are examined by means of different pathological techniques, including the use of serial sectioning and/or immunohistochemistry (IHC). A number of studies have reevaluated the lymph nodes of breast cancer patients that were thought to be negative following the initial routine histological assessment using hematoxylin and eosin (H&E) staining. Using various pathological methods, these studies found that 9–32 % of cases previously judged to be node negative contained occult micrometastases and ITC [27]. This implies that in routine pathological examinations, a similar percentage of patients may be misdiagnosed to be node negative. Although the frequency of occult metastases is relatively low, it might have a significant impact on OS and DFS. Thus, Viale et al. [28] described the exhaustive intra-operative frozen section method, in which frozen section (FS) analysis of the entire SLN is performed: 15 or more pairs of 4-μm FSs (stained with both H&E and a rapid IHC method) were analyzed until the entire node is sampled, leaving no tissue for permanent sections. The procedure, which is so laborious and takes 40–50 min, would be impossible to perform on a routine basis for many institutions.

Recently, the one-step nucleic acid amplification (OSNA) assay was developed as a rapid molecular

Table 3 Summary of studies comparing the characteristics of OSNA assay with those of pathological examinations

Author	Subjects	Sensitivity (%)	Specificity (%)	Accuracy (%)	NPV (%)	PPV (%)
Visser [32]	346ALNs	95.3	94.7	94.8	98.9	80.3
Schem [33]	343ALNs	98.1	91.7	91.8	99.1	80
Tamaki [30]	450ALNs	87.5	94.1	92.9	97.2	76.1
Feldman [34]	1044SLNs	77.5	95.8	93.4	96.6	73.8
Khaddage [35]	46Pts	80	97.2	93.5	94.6	88.9
	80SLNs	88.2	98.4	96.3	96.9	93.8
Snook [36]	194Pts	89.8	94.5	93.4	96.5	84.6
	395SLNs	91.7	96.9	95.9	98.1	86.8
Le Frere Belda [37]	233Pts	76.8	88	86.3	94.9	58.9
	503SLNs	80.9	93.9	92.2	97.2	65.4
Bernet [38]	181SLNs	89.2	95.8	94.5	97.2	84.6
Sagara [39]	61SLNs	75	98	93.4	94.1	90
Wang [40]	552Pts	87.7	89.6	89.1	95.6	73.8
	1188SLNs	83.7	92.9	91.4	96.8	69.1

ALNs axillary lymph nodes, *SLNs* sentinel lymph nodes, *NPV* negative predictive value, *PPV* positive predictive value, *Pts* patients

detection procedure targeting cytokeratin 19 (CK19) mRNA which is expressed in breast cancer cells, but not in the normal cells included in the lymph node [29, 30]. A whole assay procedure can be completed within 30-40 min, making it suitable as an intra-operative procedure for detecting SLN metastasis. The advantages of the OSNA assay include good reproducibility among institutions and the capability to examine a whole lymph node within 30–40 min. Several studies have shown that the OSNA assay is more accurate than an intra-operative pathological examination and as accurate as a post-operative examination (Table 3) [30–40]. In these studies, typically four slices are cut from each lymph node, and the two alternating slices are examined using the OSNA assay or histology. Although the sensitivity and specificity of the OSNA assay differ among the reports to some extent, these differences can be mostly explained by differences in the fineness of the histological examination among the studies, i.e., some studies [32–38, 40] adopted serial sectioning and the others did not, [30, 39]; furthermore, the differences can also be explained, at least in part, by the tissue allocation bias. Besides, recent studies have found that the amount of CK19 mRNA copy-number obtained with the OSNA assay is useful for the prediction of non-SLN involvement [41–43].

Another advantage of the OSNA method is to reduce the workload for pathologists. The OSNA assay costs 24,000 yen and the intra-operative frozen section examination costs 19,900 yen for each patient. Although the OSNA assay is slightly more expensive as a test fee, it can be performed by a laboratory technician alone unlike the frozen section examination which requires a technician for sectioning and a pathologist for diagnosis. Thus, the total cost including the test fee and labor expenses is estimated to be similar between the OSNA assay and the frozen section examination.

It is expected that the OSNA method may thus be able to overcome the issue of variability among conventional pathological examinations. Thus, a prospective study of the OSNA method using a large population and a longer follow-up may answer the question whether minimal SLN metastasis in fact affects survival. Although the current OSNA assay adopts the cutoff values of 250 copies and 5000 copies for the diagnosis of micrometastasis and macrometastasis, respectively, it is possible that the optimal cutoff value for recurrence prediction may be different from these cutoff values, and thus efforts are needed to determine the optimal cutoff value for recurrence prediction.

Conclusion

The findings of two large randomized studies, ACOSOG Z0011 and IBCSG 23-01, have demonstrated that ALND can be avoided in T1-2 N0 breast cancer patients with micrometastasis and ITC who have been treated with BCS and whole breast irradiation and who have received adjuvant chemo- and/or hormonal therapy. However, there is currently not enough evidence to omit ALND for patients with macrometastasis or those who do not meet the inclusion criteria of the ACOSOG Z0011 and IBCSG 23-01 trials (e.g., those who have undergone mastectomy). Several studies have shown substantial discrepancies regarding the prognostic significance of minimal SLN involvement. The newly developed OSNA assay may be able to overcome this variability among conventional pathological examinations.

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