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ORIGINAL ARTICLE – BREAST ONCOLOGY

Prognostic Significance of Tumor-Positive Internal Mammary Sentinel Lymph Nodes in Breast Cancer: A Multicenter Cohort Study

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ABSTRACT

Introduction. The introduction of the sentinel lymph node biopsy (SLNB) in breast cancer has renewed interest in lymphatic drainage to the internal mammary (IM) nodes. The clinical impact of tumor positive IM nodes is not completely clear. This study evaluated the incidence and impact on overall survival of metastatic IM SLNs.

Methods. Between 1997 and 2010, 3685 patients underwent surgery including SLNB for primary breast cancer following an intratumoral or peritumoral radioactive-tracer injection. The presence of lymph node metastases was categorized according to the TNM-classification. Cumulative overall survival was estimated and the influence of metastases in the IM nodes and other factors was assessed by Cox-regression-analysis.

Results. In 754 patients (20.5 %) ipsilateral IM lymph nodes were visualized on preoperative lymphoscintigraphy, retrieval rate of IM SLNs was 81.0 %. IM metastases were detected in 130 patients (21.3 % of retrieved SLNs and 3.5 % of all patients respectively). The presence of IM metastases was associated with axillary metastases ($p < 0.001$). After a median follow-up of 61.2 months, 10.9 % of patients had died. In a multivariate analysis IM metastases did not have a significant effect on overall survival [HR] 1.20; CI: 0.73–1.98. In patients without axillary metastases ($n = 2398$), the presence of IM metastases ($n = 43$) was associated with worse survival [HR] 2.68; 95 % CI: 1.30–5.54.

Conclusion. Overall, the presence of IM metastases did not effect overall survival independent of other prognostic factors including axillary metastases. However, the small subgroup of patients who had IM metastases alone had worse outcome than patients without any regional lymph node metastases.

Historically, internal mammary (IM) lymph node metastases were associated with an unfavorable prognosis in breast cancer patients.[1,2] This observation stems from the era when IM lymph nodes were dissected as part of an extended mastectomy. Today, IM lymph node dissection is not performed in breast cancer patients as it causes substantial morbidity and fails to contribute to locoregional control or overall survival (OS).[2]

Introduction of the sentinel lymph node biopsy (SLNB) in breast cancer patients offered the opportunity for a more targeted surgical approach to the IM chain. Depending on the method of radioactive tracer injection, drainage to the IM sentinel lymph node (SLN) is observed in 13–37 % of patients, among whom only 8–24 % have metastases.[3]

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Although the need to harvest these IM SLNs is controversial, it can be performed with minimal morbidity.[4,5]

Observation of IM SLNs has renewed interest in the prognostic relevance of IM lymph node metastases. A number of studies have addressed the clinical impact of IM metastases in terms of additional treatment.[4,6,7] The present study adds to this knowledge with its evaluation of the prognostic impact of lymph node metastases in harvested IM SLNs.

PATIENTS AND METHODS

Between February 1997 and November 2010, a total of 4232 patients in three hospitals (Diakonessenhuis Utrecht/Zeist (A), The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (B), and Orbis Medical Centre, Sittard (C) underwent surgical treatment including SLNB for primary cT1-2N0 breast cancer. Data regarding the operative procedures were collected prospectively. Ultimately excluded were 12 men with in situ carcinoma ($n = 121$), patients with a history of previous breast cancer or other malignancies ($n = 200$ and $n = 68$, respectively), patients with a synchronous, contralateral breast cancer (53 patients, 106 tumors), and patients who had received neoadjuvant chemotherapy ($n = 44$). One patient was lost to follow-up immediately after the operation.

Lymphoscintigraphy and Surgery of SLNs

Lymphoscintigraphy protocols contained discrete differences but consistently included intratumoral or peritumoral injection of ^{99m}Tc nanocolloid. One hospital used a 1-day protocol and the other institutions a 2-day protocol. There were differences in the administered ^{99m}Tc doses.[4,6,8] Intraoperatively, a peritumoral injection of patent blue dye (Bleu patenté V; Laboratoire Guerbet, Aulnay-sous-Bois, France) was used for SLN identification. Visualization rates have been published previously for the three institutions (22, 22, and 20 % in hospitals A, B, and C, respectively).[4,6,8] Axillary SLNs were retrieved first. When no axillary SLN was visualized on preoperative lymphoscintigraphy, the axilla was explored in search of an SLN containing the blue dye. Subsequently, we evaluated the patient for visually identified IM SLNs. A γ-ray detection probe was used to guide a parasternal intercostal incision. Partial rib resection was not required to retrieve IM lymph nodes. In addition to retrocostal localization of an IM SLN, the impossibility of discerning radioactivity of the SLN from the background activity following intraparenchymatous tracer injection was a main reason why IM SLNs could not be retrieved in these institutions.[4,6,8]

Pathology

The number of sections of a lymph node and distance between the cuts varied. In hospital A, bisected axillary SLNs were formalin-fixed and cut at five levels with intervals of 250 μm. Because IM SLNs were usually too small to bisect, they were processed as a whole and sectioned at five levels. In hospital B, bisected SLNs and IM SLNs were formalin-fixed, embedded in paraffin, and cut at a minimum of six levels at 50- to 150-μm intervals. In hospital C, the SLNs were formalin-fixed and bisected if large enough, with five cuts at 100-μm intervals. At all three hospitals, pathological evaluation of all SLNs consisted of hematoxylin-eosin and immunohistochemical cytokeratin-8 staining.

Primary tumor characteristics were also noted. Estrogen (ER) and progesterone (PR) receptor status and the Bloom–Richardson (BR) malignancy grade of the primary tumor were determined throughout the study period. Beginning in 2004, the HER2 receptor status was routinely assessed. The presence of metastases in axillary and IM lymph nodes and the number of involved metastatic lymph nodes were recorded. Lymph node status was classified according to the International Union Against Cancer TNM classification, 7th edition.[9]

Postoperative Treatment

Patients received adjuvant systemic therapy based on Dutch guidelines. These guidelines were adjusted several times during the study period, resulting in an increasing proportion of patients with node-negative disease that was a result of systemic therapy. Locoregional radiotherapy was indicated in patients with four or more metastatic axillary lymph nodes. In patients with IM metastases and none to three tumor-positive axillary lymph nodes, parasternal irradiation was advised.

Follow-Up

The last patient included in our study for hospital A was treated in November 2010, for hospital B in June 2006, and for hospital C in August 2010. Follow-up for hospital A was conducted until January 2011. The local databases of hospitals B and C were merged with The Netherlands Cancer Registry (NCR). This database contains information on patients' vital status through linkage with data of the municipal personal records database, which has complete information on all deceased and emigrated residents of The Netherlands. Vital status was complete up to February 1, 2010.

TABLE 1 Baseline characteristics according to IM lymph node status of 3685 patients with cT1-2N0 breast cancer operated on in three Dutch hospitals between 1997 and 2010

Characteristics	IM-negative (n = 3555, 96 %)	IM-positive (n = 130, 4 %)	p*
Patients			
Age at surgery (years), median (min–max)	58 (24–96)	50 (32–85)	<0.001**
≤50 Years	888 (25 %)	66 (51 %)	<0.001
>50 Years	2667 (75 %)	64 (49 %)	
Missing	0	0	
Tumors			
Axillary status			
Node-negative	2355 (66 %)	43 (33 %)	<0.001
Node-positive	1200 (34 %)	87 (67 %)	
Missing	0	0	
Tumor size			
pT1	2379 (67 %)	76 (58 %)	0.078
pT2	1121 (32 %)	51 (39 %)	
pT3	47 (1 %)	3 (2 %)	
Missing	8	0	
Tumor grade			
1	1222 (35 %)	39 (31 %)	0.58
2	1458 (41 %)	54 (43 %)	
3	836 (24 %)	34 (27 %)	
Missing	39	3	
Hormone receptor status			
Negative	572 (16 %)	20 (16 %)	0.9
Positive	2909 (84 %)	108 (84 %)	
Missing	74	2	
HER2 status			
Negative	2263 (87 %)	85 (87 %)	1.0
Positive	345 (13 %)	13 (13 %)	
Missing	947	32	
Treatment			
Surgical procedure			
Breast-conserving	2114 (59 %)	74 (57 %)	0.59
Mastectomy	1441 (41 %)	56 (43 %)	
Missing	0	0	
Radiotherapy			
No	1189 (34 %)	17 (13 %)	<0.001
Yes	2346 (66 %)	112 (87 %)	
Missing	20	1	
Adjuvant chemotherapy			
No	2356 (67 %)	40 (31 %)	<0.001
Yes	1172 (33 %)	89 (69 %)	
Missing	27	1	
Adjuvant hormonal therapy			
No	2152 (61 %)	34 (27 %)	<0.001
Yes	1372 (39 %)	93 (73 %)	
Missing	31	3	

TABLE 1 continued

Characteristics	IM-negative ($n = 3555$, 96 %)	IM-positive ($n = 130$, 4 %)	p*
Adjuvant trastuzumab			
No	3465 (97 %)	125 (96 %)	0.39
Yes	90 (3 %)	5 (4 %)	
Missing	0	0	

IM internal mammary

* Fisher's exact test, except for ** Mann–Whitney *U* test

Statistical Analysis

Baseline characteristics between patients with and without IM lymph node metastases for relevant prognostic clinicopathological factors were compared using Fisher's exact tests for categorical data and Student's *t* tests or the Mann–Whitney *U* tests for continuous data (Table 1). We then used Cox proportional hazard analyses to assess the relation between IM metastases and OS. Follow-up started at the date of the operation and ended with death (event) or with the date of last follow-up (censored). We defined multiple Cox models by adjusting the possibly confounding effects of IM status for an increasing number of clinicopathological factors: model 1 was adjusted for age (continuous). Model 2 was additionally adjusted for year of diagnosis (continuous), tumor size (pT2 and pT3 versus pT1), Bloom–Richardson (BR) grade (grades 2 and 3 vs. 1), ER+ and/or PR+ (yes/no), HER2 status (±), and number of axillary lymph node metastases (continuous). Model 3 was additionally adjusted for type of surgery (mastectomy versus breast-conserving therapy) as well as adjuvant radiotherapy, trastuzumab treatment, hormonal treatment, and chemotherapy (yes/no for the latter four factors). As patients were treated in three hospitals, this clustering was taken into account in all models by including a random effect for each hospital using a frailty approach. Age and the number of axillary lymph node metastases were modeled using restricted cubic spline functions as they showed significant nonlinearity with OS [based on the likelihood ratio (LR) test compared to fully adjusted models with only the linear term]. The proportionality assumption was checked and found not violated by inspecting the Schoenfeld residuals for all variables. We performed subgroup analyses for patients with and without axillary metastases and for patients treated with mastectomy and with breast-conserving therapy. We then statistically tested for differential effects using interaction terms between IM status and the subgroups (LR tests). We also repeated the analyses considering tumor deposits <0.2 mm in IM SLNs (isolated tumor cells) as IM metastasis-negative.

Not all patients had complete data. HER2 status was not routinely determined before 2004, so it was not available for 27 % of patients. Other variables were complete for >98 % of cases. Missing values were multiply imputed,[10–12] and results were pooled.[13,14] Data were analyzed in R software, version 3.0.1 (R Tech Solutions, Kolkata, India). All reported p values were two-sided with a 5 % threshold for statistical significance.

RESULTS

SLNs were visualized using lymphoscintigraphy in 3606 of the 3685 patients (98 %). In all, 2852 patients (79 %) had axillary SLNs, 703 (20 %) had axillary and IM SLNs and 51 (1.4 %) had only IM SLNs on lymphoscintigraphy. SLNs were retrieved in 3640 patients (99 %). Only axillary SLNs were removed from 3029 patients (83 %), axillary and IM SNLs were removed from 584 patients (16 %), and only IM SLNs were removed from 27 patients (0.7 %). The retrieval rate of IM SLNs was 81.0 %.

Pathology evaluation revealed axillary metastases in 1287 patients (35 %) and IM metastases in 130 patients (21.0 % of retrieved SLNs—3.5 % of all patients). Extrapolating the metastatic rate (21 %) to the 143 patients in whom IM SLNs were visualized but could not be retrieved implied an additional unidentified 30 patients with IM metastases and an expected overall percentage of metastatic IM SLNs in 4.3 % in all patients. Among the 130 patients with IM SLNs, 14 had isolated tumor cells in the IM SLN. Women with IM metastases were significantly more likely to be younger and more often had axillary lymph node involvement than patients without IM metastases (67 vs. 34 %; $p < 0.001$). In the group with IM metastases, only 43 patients had metastatic IM lymph nodes (Table 1). Patients with IM metastases were significantly more likely to have been exposed to radiotherapy and adjuvant hormonal or chemotherapy (89, 67, and 73 %, respectively; $p < 0.001$).

TABLE 2 Overall survival for 130 patients with cT1-2N0 breast cancer with IM metastases (from three Dutch hospitals operated on between 1997 and 2010) under various adjustment conditions

Parameter	Unadjusted		Age-adjusted		Clinicopath. adjusted, with HER2[a]		Full adjustment including adjuvant treatment	
	HR (95 % CI)	*p*	HR (95 % CI)	*p*	HR (95 % CI)	*p*	HR (95 % CI)	*p*
IM positive	1.05 (0.64–1.71)	0.85	1.27 (0.78–2.08)	0.33	1.11 (0.68–1.83)	0.68	1.20 (0.73–1.98)	0.48
Age at diagnosis (nonlinear)[b]	–	–	–	<0.001	–	<0.001	–	<0.001
Year of diagnosis (per year)	–	–	–	–	0.95 (0.91–1.00)	0.035	0.96 (0.92–1.00)	0.08
No. of positive axillary lymph nodes (nonlinear)[b]	–	–	–	–	–	<0.001	–	<0.001
pT2	–	–	–	–	1.62 (1.30—2.02	<0.001	1.69 (1.34–2.12)	<0.001
pT3	–	–	–	–	2.51 (1.43–4.41)	0.001	2.69 (1.50–4.82)	0.001
Bloom Richardson grade 2	–	–	–	–	1.53 (1.15–2.03)	0.003	1.57 (1.18–2.09)	0.002
Bloom Richardson grade 3	–	–	–	–	1.87 (1.36–2.59)	<0.001	2.09 (1.50–.91)	<0.001
ER- and/or PR-positive	–	–	–	–	0.56 (0.43–0.72)	<0.001	0.56 (0.41–0.77)	<0.001
HER2-positive	–	–	–	–	1.00 (0.72–1.39)	0.99	1.03 (0.74–1.44)	0.85
Adjuvant chemotherapy	–	–	–	–	–	–	0.68 (0.51–0.90)	0.007
Adjuvant hormonal therapy	–	–	–	–	–	–	0.91 (0.69–1.21)	0.53
Adjuvant trastuzumab	–	–	–	–	–	–	0.44 (0.06–3.29)	0.43
Radiotherapy	–	–	–	–	–	–	0.92 (0.66–1.30)	0.65
Mastectomy	–	–	–	–	–	–	1.03 (0.74–1.42)	0.88

Clustering for different hospitals was adjusted by including a random effect for the hospital using a frailty approach

Clinicopath. clinicopathologically, *HR* hazard ratio, *CI* confidence interval, *ER* estrogen receptor, *PR* progesterone receptor

[a] Data on HER2 status were imputed in 27 % of patients

[b] Age and number of axillary lymph nodes were modeled using restricted cubic spline functions

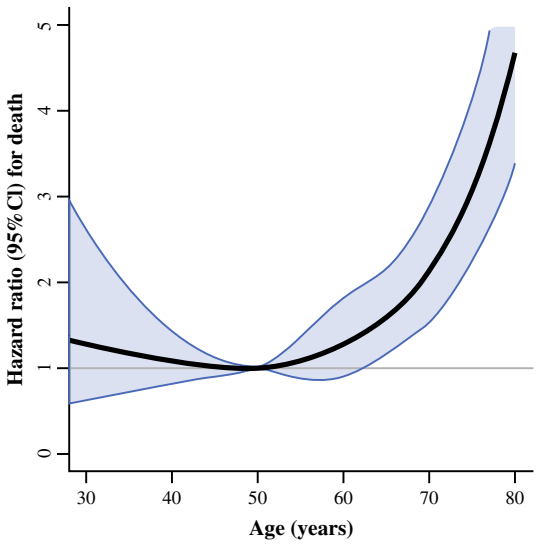

FIG. 1 Overall survival for cT1-2N0 breast cancer patients according to age (continuous) on the basis of Cox proportional hazard analyses fully adjusted for clinicopathological factors—e.g., tumor size, Bloom–Richardson (BR) grade, receptor status—and adjuvant treatment. *CI* confidence interval

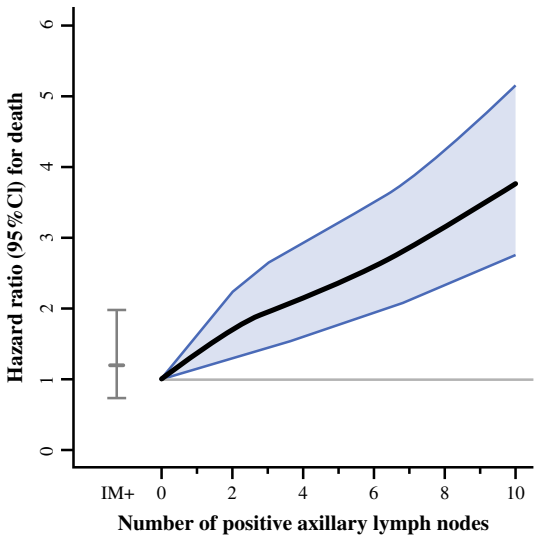

FIG. 2 Overall survival for cT1-2N1-2 breast cancer patients according to the number of positive axillary lymph nodes on the basis of Cox proportional hazard analyses fully adjusted for clinicopathological factors (e.g., tumor size, BR grade, receptor status), and adjuvant treatment

Patient Outcomes

After a median follow-up of 61 months (0.1–163 months), 3264 women were still alive (88.6 %). Altogether, 403 patients (10.9 %) had died, and 18 (0.5 %) were lost to follow-up before February 1, 2010. Of the patients with IM metastases, 17 (13.1 %) had died.

After adjustment for age differences (model 1), patients with IM metastases had a 27 % higher risk of dying than patients without IM metastases, albeit the difference was not [hazard ratio (HR) 1.27, 95 % CI 0.78–2.08)]. This result remained after full adjustment for clinicopathological and treatment factors (HR 1.20, 95 % CI 0.73–1.98) (Table 2). Although considering the 14 patients with isolated tumor cells in IM SLNs as IM metastasis-negative led to a higher risk estimate (HR 1.30, 95 % CI 0.77–2.19; fully adjusted), it also was not statistically significant.

The relation between IM status and OS depended on the presence of axillary metastasis in our data (p for interaction <0.001). Among the patients without axillary metastases ($n = 2398$), 43 had IM metastases, and they had a higher risk of dying (HR 2.68, 95 % CI 1.30–5.54; $p = 0.008$; fully adjusted) than patients without IM metastases in this group. When axillary metastases were present, there was no relation of IM metastases with outcome for the 1200 IM-negative patients (HR 0.79, 95 % CI 0.40–1.57; $p = 0.51$; fully adjusted). There were eight deaths among the 87 IM-positive patients and 190 deaths.

The relations between other clinicopathological factors and OS are shown in Table 2. Especially tumor size and BR grade increased the risk of dying, whereas patients with a hormone receptor-positive tumor were at lower risk. The nonlinear relation between age and OS is shown in Fig. 1. The HR for dying increased steeply with each additional axillary metastasis up to two, after which the risk still increased but less strongly (Fig. 2). In comparison to the risk of axillary lymph node involvement, the absolute HR of IM metastases, albeit statistically nonsignificant, approximated the risk of less than one involved axillary metastasis.

DISCUSSION

In this multicenter cohort of patients staged by SLNB using an intraparenchymal tracer injection, 36.0 % of the patients had metastases in the regional lymph nodes, and 3.5 % had metastases in the IM chain. In terms of OS, IM SLN metastases did not have a significant prognostic impact independent of other clinicopathological factors, including axillary metastases. The subgroup of patients without axillary metastases had a worse outcome than those with uninvolved regional lymph nodes.

The main strengths of this study are its multicenter approach and the relatively large cohort of patients with IM metastases. With more than 100 patients having IM lymph node metastases, the present study describes the largest cohort of patients with IM node metastases to date. SLNB procedures were comparable with respect to the use of an intraparenchymal nanocolloid injection at all three hospitals. It is well known that this technique is associated with a higher rate of visualizing IM SLNs,[15–17] which was the reason for pooling the data of these particular institutions in the first place. The long time frame during which we

collected data on breast cancer patients also implied that there have been changes in confounding factors. Because the proportion of patients receiving adjuvant treatment increased during the study period owing to guideline changes over the years, we adjusted for the year of diagnosis as well as other potential confounders. Visualization and retrieval rates for IM SLNs have been reported previously.[16,18,19] The retrieval rate in the present study was 81 %, although not all IM metastases were likely identified as such. This potentially led to an underestimation of the true relation between IM metastases status and OS as these unrecognized IM metastases were misclassified as IM-negative. It is unlikely that this misclassification is related to the outcome (i.e., random misclassification).

Although we did not find that IM metastases has a statistically significant independent effect on OS in the present study, this finding is in contrast to earlier reports. IM metastases were considered a poor prognostic sign in earlier times. In a landmark study by Veronesi et al.[20] patients with metastases in the IM chain alone had a prognosis similar to that of patients with axillary metastases, and patients with both axillary and IM metastases had the poorest prognosis. A comprehensive review also showed that metastases in the IM lymph nodes added to the prognostic impact of the status of other regional lymph nodes.[21] Patients with IM lymph node metastases were then classified as pN3.[22] The gloomy prognosis associated with IM metastases in earlier times contrasts with the insignificant influence observed in the present study. After multivariate adjustment for systemic therapy, the relative risk of death associated with IM metastases was HR 1.20. Albeit not significant, in absolute terms it is comparable to a relative risk increase in the presence of one involved axillary node, as shown in Fig. 1. The adverse prognostic impact of IM metastases in patients who have uninvolved axillary lymph nodes is in line with findings from the aforementioned studies. All in all, metastases in IM SLNs are better regarded as "just" another regional lymph node than considering it as a staged category in itself.

The SLNB offers a minimally invasive, targeted approach to determine IM lymph node status. Even though the present study did not show a significant prognostic influence of IM metastasis, the subgroup of patients without axillary metastasis but with IM metastasis did have a worse outcome than patients with uninvolved regional lymph nodes. Therefore, not addressing IM lymph node status could lead to understaging.

The introduction of systemic treatment is a potential confounder and has had a major impact on survival rates for all breast cancer stages since the time that IM node dissections were abandoned. In the present cohort of breast cancer patients, the 5-year OS was approximately 90 %, with half of the deceased patients having died from other causes. Although systemic therapy has influenced the absolute survival rates, it cannot be the sole explanation for the absence of a significant prognostic impact of IM metastases. We therefore tried to adjust for the use of systemic treatment. A certain degree of patient selection persisted, however, so full adjustment for confounding remains difficult to achieve. Our results should be interpreted from that perspective.

A likely explanation for the statistical and clinical prognostic irrelevance of IM metastases lies in the SLNB procedure itself. First, IM lymph nodes are smaller than axillary nodes and are thus unlikely to be detectable by means other than an SLNB procedure. Consequently, IM nodes retrieved by SLNB reflect a different selection than IM lymph nodes harvested during earlier times. In addition, the current pathological workup of SLNs reveals smaller tumor deposits. The 10 % of patients with IM lymph node involvement in the present study who had deposits <0.2 mm (isolated tumor cells) underscores this retrieval of smaller IM metastases during the SLNB era. Our study supports considering IM metastasis as a "variety" of regional lymph node involvement. Thus, the presence of IM metastases, in prognostic terms, equates to a single involved axillary node.

The aim of this study was to determine the impact of IM lymph node metastases on the prognosis of breast cancer patients. In this large cohort study IM metastases were found in a considerable proportion of patients, but we did not observe an overall impact of IM lymph node metastases on OS, independent of axillary metastases and other clinicopathological factors. Only 1 % of all of the patients who had IM metastases—but otherwise uninvolved regional lymph nodes—had significantly impaired prognosis. Then again, previous studies demonstrated that the detection of these IM node metastases altered nonsurgical treatment in a larger proportion of patients. Hence, we advise that SLNB of the IM nodes be performed for optimal staging of the breast cancer, at least in patients who will not undergo adjuvant systemic treatment based on the primary tumor's characteristics. Concomitantly, we consider parenchymatous tracer injection as the preferable technique for optimizing visualization of IM SLNs.

APPENDIX

Lymphoscintigraphy Protocols

In hospital A, lymphoscintigraphy was performed on the day of surgery. Patients received a combination of peritumoral intraparenchymal and subcutaneous injections around and ventral of the tumor of an average dose of 77.6 MBq (spread 53–150 MBq) ^{99m}Tc-nanocolloid (Nanocoll, GE Health). The total volume was 0.6 mL

nanocolloid in physiologic saline, given in 2–4 equal doses. In case of non-palpable breast tumors injections were guided by using a 7.5 MHz ultrasound probe (Aloka). After injection the area was massaged gently until the appearance of the SLN. Semi-dynamic images were performed at the initial visualization time of the lymphatic channel. Static images were obtained approximately 2 h after injection depending on the time of surgery. Semi-dynamic and static images were obtained during a 2 min imaging time on the Toshiba 901 HG single-head gamma camera, using low energy high resolution collimators between June 1999 and October 2005. Since November 2005 the images were performed on the Philips skylight dual head gamma camera, using low energy general purpose collimators. The images were performed with a ^{57}Co flood source. A skin marker was placed on the projection of the SN using a handheld γ-ray detection probe (Europrobe, PI Medical diagnostic equipment BV).

In hospital B a 2-day protocol was used. On the day before surgery, ^{99m}Tc-labeled nanocolloid (Nanocoll; Amersham Cygne, Eindhoven, The Netherlands) was injected into the lesion in a mean volume of 0.2 mL and a mean radioactivity dose of 114.9 MBq (3.1 mCi). In case of nonpalpable breast cancer, the intratumoral injection was guided by ultrasound or stereotaxis. Static imaging was performed at 30 min and 4 h after injection with simultaneous transmission scanning by using a cobalt-57 flood source to outline the body contour. Since July 1999, additional views were obtained after 2 h. Both anterior and lateral images were obtained by using a dual-head gamma camera (Vertex; ADAC, Milpitas, CA). The location of the node was marked on the skin with indelible ink. In patients with nonpalpable breast cancer, a localization procedure was performed after the last scintigraphic image, including placement of a catheter for intratumoral administration of patent blue dye.[6] In hospital C, the injection of 10 mCi (370 MBq) ^{99m}Tc-nanocolloid, the day before surgery in 3–4 depots around the tumor or in the breast parenchyma surrounding the cavity of a previous excisional biopsy. In case of non-palpable tumors, the radiocolloid tracer was injected within the relevant quadrant of the breast, without the use of ultrasound guidance. Lymphoscintigraphy was performed on the next day, after a period of 16–18 h following radiotracer injection, and shortly before surgery. Lymphoscintigraphic images were obtained in three standard positions: anterior, anterior oblique and lateral. The location of axillary and non-axillary SNs was marked on the skin. After induction of general anesthesia in the operating room, 10–15 min before the incision, 0.8–1.0 ml Patent Blue V (Laboratoire Guerbet, France) was injected intradermally above the tumor or alongside the scar of the excisional biopsy.[8]

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