

# REPORTS

## Sentinel Lymph Node Biopsy and Axillary Dissection in Breast Cancer: Results in a Large Series

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**Background:** Axillary lymph node dissection is an established component of the surgical treatment of breast cancer, and is an important procedure in cancer staging; however, it is associated with unpleasant side effects. We have investigated a radioactive tracer-guided procedure that facilitates identification, removal, and pathologic examination of the sentinel lymph node (i.e., the lymph node first receiving lymphatic fluid from the area of the breast containing the tumor) to predict the status of the axilla and to assess the safety of foregoing axillary dissection if the sentinel lymph node shows no involvement. **Methods:** We injected 5–10 MBq of  $^{99m}\text{Tc}$ -labeled colloidal particles of human albumin peritumorally in 376 consecutive patients with breast cancer who were enrolled at the European Institute of Oncology during the period from March 1996 through March 1998. The sentinel lymph node in each case was visualized by lymphoscintigraphy, and its general location was marked on the overlying skin. During breast surgery, the sentinel lymph node was identified for removal by monitoring the acoustic signal from a hand-held gamma ray-detecting probe. Total axillary dissection was then carried out. The pathologic status of the sentinel lymph node was compared with that of the whole axilla. **Results:** The sentinel lymph node was identified in 371 (98.7%) of the 376 patients and accurately predicted the state of the axilla in 359 (95.5%) of the patients, with 12 false-negative findings (6.7%; 95% confidence interval = 3.5%–11.4%)

among a total of 180 patients with positive axillary lymph nodes. **Conclusions:** Sentinel lymph node biopsy using a gamma ray-detecting probe allows staging of the axilla with high accuracy in patients with primary breast cancer. A randomized trial is necessary to determine whether axillary dissection may be avoided in those patients with an uninvolved sentinel lymph node. [J Natl Cancer Inst 1999;91:368–73]

For a century, axillary dissection has been an essential component of the surgical treatment of infiltrating breast cancer. However, following the development of imaging techniques that detect small, early stage primary carcinomas, it is increasingly noted that axillary dissection finds only healthy lymphatic nodes (1–3). Furthermore, it has been suggested that axillary dissection should not be viewed as a curative but mainly a staging procedure for obtaining as much prognostic information as possible (4,5).

The question, therefore, is whether lymph node removal is always necessary in patients with a clinically negative axilla. The ideal solution would be to diagnose lymph node involvement through noninvasive techniques, but this seems unrealistic at present (6). A promising alternative appears to be sentinel lymph node biopsy: this involves the identification, removal, and pathologic examination of the lymph node that first receives lymph from the area of the breast containing the tumor. The assumption is that if the sentinel lymph node is negative, all other axillary lymph nodes will be negative, and this implies orderly spread of breast cancer cells within the axilla—a phenomenon that is well established (7,8). Sentinel lymph node biopsy was first used in melanoma with encouraging results (9–11). Studies (12–14) on its use in breast cancer have provided positive results, but definitive indications and contraindications for the technique have not been established.

We designed this study with the aim of clarifying the role of sentinel lymph node biopsy in the clinical management of breast cancer and identifying indications for the use of the technique. In a series of patients with breast cancer, we compared sentinel lymph node status with the de-

finite histologic result of all axillary lymph nodes after complete axillary dissection.

## PATIENTS AND METHODS

### Patients

We studied 376 consecutive patients with operable breast carcinoma enrolled at the European Institute of Oncology during the period from March 1996 through March 1998. All patients were scheduled to receive total axillary dissection according to established criteria (7,8) and all gave their informed consent to receive sentinel lymph node biopsy. Findings from 163 of these patients have been previously reported (14).

Reasons for exclusion were pregnancy, lactation, noninfiltrating carcinoma, and previous excision biopsy of the breast. Also excluded were patients with clinically evident metastatic involvement of the axilla. In five patients, no lymphatic drainage pattern was revealed by lymphoscintigraphy performed to locate the sentinel lymph node; all subsequent data refer to the 371 patients in whom the lymph node was revealed by lymphoscintigraphy and the gamma ray-detecting probe. The average age of the patients was 52 years (range, 25–77 years). Tumor size was less than 1.5 cm in 142 (38.3%) patients, between 1.5 and 1.9 cm in 115 (31%) patients, and greater than 1.9 cm in 114 (30.7%) patients. One hundred eighty-nine tumors were in the right breast (107 upper outer, 32 upper inner, 31 lower outer, 13 lower inner, and six in the central quadrants), while 182 were in the left (99 upper outer, 34 upper inner, 26 lower outer, 12 lower inner, and 11 in the central quadrants). Mean pathologic tumor diameter was 1.7 cm. Three hundred forty-two patients received quadrantectomy, axillary dissection, and subsequent radiotherapy to the breast, and 29 received a modified radical mastectomy.

### Scintigraphic Identification of the Sentinel Lymph Node

The day before surgery, 5–10 MBq of  $^{99m}\text{Tc}$ -labeled colloidal particles of human albumin (size range, 200–1000 nm) in 0.2 mL of saline were injected close to the tumor (15,16) (Albures; Sorin Biomedica, Saluggia, Italy). Injection was subder-

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mal if the tumor was superficial and peritumoral if it was deep. Lymphoscintigraphy was then performed (the same day) to reveal the dynamics of lymphatic flow; this was particularly useful if more than one lymph node showed uptake of tracer. Anterior and anterior-oblique projections of the breast and axilla were obtained to determine the exact position of the sentinel lymph node. The skin projection of the lymph node was then marked and used as a landmark when beginning the dissection.

### Localization and Removal of Sentinel Lymph Node

Sentinel lymph node biopsy usually took place 14–20 hours after injection of radiolabeled albumin. A gamma ray-detecting probe (Scinti Probe: MR 100, Pol.Hi.Tech, L'Agua, Italy) in a sterile glove was employed to locate the radioactive lymph node and facilitate its removal. Two such probes are in use at our Institute, they are checked weekly and calibrated so that peak sensitivity is at 140 keV, and the window is set to 125–150 keV. Before incision, the probe was passed slowly over the marked skin area to locate the lymph node. Radioactivity detected by the probe was transduced into digital read-out and acoustic signals whose intensity was directly proportional to the level of radioactivity. A 2–3-cm incision was then made and the lymph node was identified and isolated using the probe as a guide. Total axillary dissection was then performed, removing all lymph nodes, including those at the third level as originally defined by Berg (17). The dissection began by extending medially and laterally the biopsy incision and carefully retracting the skin upward.

The mean number of lymph nodes removed was 26 (range, 10–54 lymph nodes): 15 at the first level, six at the second, and five at the third (17). The first level includes all lymphatic tissue lateral to the lateral margin of the pectoralis minor muscle, the second level includes lymph node behind the pectoralis minor muscle, and the third level is the apex of the axilla. All removed material was checked in the operating room for radioactivity before being sent for pathologic examination.

In a consecutive subgroup of 54 patients, we performed sentinel lymph node biopsy using both radiolabeled albumin and blue dye. The technique of albumin injection was the same as described above. Immediately after induction, 5 minutes before skin incision, 4 mL of blue dye was injected subdermally or peritumorally, depending on the depth of the tumor. The incision was made as normal to remove the sentinel lymph node. The axillary area was inspected for both blue-dye staining and radioactive nodes, which were removed.

### Pathology

In the first 60 patients, the sentinel lymph nodes identified by the gamma ray-detecting probe, and the remaining axillary lymph nodes, were examined by standard histology. In the remaining 311 patients, sentinel lymph nodes were sent for frozen-section examination; 192 of these were examined by the usual technique of lymph node bisection followed by freezing of half the lymph node and examination of at least three serial sections. The remaining frozen tissue was thawed, fixed, and embedded to produce permanent sections. Standard histologic examination was performed subsequently.

Preliminary analysis revealed a relatively frequent discordance between the frozen-section findings and the definitive histologic results. We therefore introduced a new and more extensive intraoperative frozen-section evaluation in September 1997 and applied it to the last 119 patients of the series. The fibrous-fatty tissue surrounding the lymph node was carefully removed without breaking the capsule. The lymph node was then bisected along its major axis. Both halves were embedded in medium used for freezing the tissues (Cellpath, Hemel Hempstead, England) with cut surfaces up and tissues frozen in isopentane cooled with liquid nitrogen. Lymph nodes less than 5 mm were embedded and frozen uncut. If two or more sentinel lymph nodes were sent, all were examined.

Fifteen pairs of frozen sections, 4  $\mu$ m thick, were cut at 50- $\mu$ m intervals, amounting to around 60 sections per lymph node (30 sections per half lymph node). If residual tissue was left, additional pairs of sections were cut at 100- $\mu$ m intervals until the lymph node was completely sampled. One section of each pair was stained with hematoxylin–eosin; if this traditional stain was negative or doubtful, the other was stained for cytokeratins using a rapid method (EPOS Anti-cytokeratins/HRP; Dako, Copenhagen, Denmark) involving the MNF116 monoclonal antibody.

Tumors were classified histologically according to the World Health Organization Histological Classification of Breast Tumors (18), as modified by Rosen and Obermann (19). Tumor grading was assessed according to Elston and Ellis (20). We looked for peritumoral vascular invasion as recommended by Rosen and Obermann (19).

The other axillary lymph nodes were isolated from fat tissue without freezing or preservation and examined by standard technique. Lymph nodes greater than 0.5 cm were bisected; lymph nodes smaller than 0.5 cm were fixed and embedded uncut. Three sections were obtained from each lymph node at different levels (100–500  $\mu$ m apart) and stained with hematoxylin–eosin.

Estrogen and progesterone receptor status and proliferative index were assessed immunohistochemically on paraffin sections of the primary tumor using an indirect avidin–biotin–peroxidase staining system (21). Following antigen retrieval with citrate buffer (0.01 M, pH 6.0) in a microwave oven (22), immunostaining was carried out in an automatic immunostainer (Techmate 500; Dako-Biotech, Glostrup, Denmark). Primary monoclonal antibodies to

estrogen receptors and progesterone receptors (Dako, Glostrup, Denmark) were used at 1/100 dilution, and MIB-1 monoclonal antibody to the Ki-67 antigen (Immunotech, Marseilles, France) was used at 1/200 dilution.

### Radiation Protection

Patients injected with radioactivity can be a source of contamination for the operating room, surgical instruments, and personnel. Furthermore, the surgically removed tissue still contains residual activity (<1.85 MBq) 16 h after injection. The staff involved in the procedure (surgeons, nurses, and pathologist) are only exposed to external radiation, since skin contamination is prevented by gloves and gowns.

We assessed residual activity in removed tissue and contamination of surgical instruments in a series of 100 consecutive operations by means of a sodium iodide well counter and thermoluminescent dosimeters, respectively. Mean residual activity in excised sentinel lymph nodes was  $9 \times 10^{-3}$  (range,  $0.7 \times 10^{-3} - 15 \times 10^{-3}$ ) MBq and 0.9 (range, 0.4–1.1) MBq in removed breast tissue.

The doses absorbed by staff in 100 operations are shown in Table 1 in comparison to the recommended dose limits for the general population according to International Commission on Radiological Protection (ICRP) recommendations (23) and Euratom Directive 96/29 (24). Considering that each surgical team performs an average of 50 operations involving sentinel lymph node biopsy each year, these personnel can therefore be classified as non-exposed workers as defined by the ICRP and Euratom.

### Statistical Methods

The 95% confidence intervals (CIs) for percentages were calculated using the exact method based on the binomial distribution (25). The associations between axillary involvement and tumor categories were evaluated by chi-squared tests. In small samples, exact *P* values were calculated using permutation tests (26). A logistic regression model with backward variable selection was used to examine the joint effect of tumor characteristics on nodal involvement. The variables for which the likelihood ratio chi-squared statistic did not indicate a significant contribution to the model were omitted (*P*<.05). Goodness of fit of the model was assessed by a likelihood ratio test compared with the saturated

Table 1. Total absorbed doses for clinical staff in 100 consecutive breast cancer operations

Operator	Total absorbed dose, $\mu$ Sv, in 100 operations*	Annual dose limits, $\mu$ Sv, for the general population recommended safe by ICRP†
Surgeon, operating room nurse: hands	450 $\pm$ 20	50 000
Pathologist: hands	75 $\pm$ 3	
Surgeon: lens of the eye	110 $\pm$ 30	15 000
Pathologist: lens of the eye	15 $\pm$ 5	
Surgeon: effective dose	90 $\pm$ 25	1 000
Pathologist: effective dose	15 $\pm$ 4	

\*Mean values and standard deviations were obtained from the air Kerma rate near patients or tissue specimens and the times required to carry out the different tasks. Approximately 50 of sentinel lymph node operations are performed by each surgeon per year; for nurses, the figure is around 30.  $\mu$ Sv = micro Sieverts.

†International Commission on Radiological Protection (ICRP) Publ. No. 60, 1990 (24).

model. Fisher's exact test was used to investigate the association between the false-negative rate and the prognostic factors. All of the statistical analyses were carried out using Splus 3.3. (1995) (Mathsoft, StatSci; Advanced Data Analysis Software, Seattle, WA). All statistical tests were two-sided.

## RESULTS

### Sentinel Lymph Node Identification

The sentinel lymph node was identified in 371 (98.7%) of the 376 patients. Lymphoscintigraphy did not reveal the lymphatic system in five patients and it was therefore not possible to mark the position of the lymph node on the skin. The next day, the probe also failed to locate a lymph node. Most of the radioactivity remained in the breast. In the remaining 371 patients, tracer uptake was sufficient to identify the sentinel lymph node during surgery. However, uptake varied greatly from 10 to 2000 counts per second. In the majority of these patients (249 [67.1%] of 371), only one sentinel lymph node was found by the probe. In 97 (26.1%) patients, two lymph nodes were radioactive, although with different levels of uptake; in 24 (6.5%) patients, three lymph nodes took up radioactivity and in one (0.3%) patient, four lymph nodes were found (Table 2). All radioactive lymph nodes detected by the probe were removed and labeled sentinel lymph nodes.

A sentinel lymph node was found in each of the first 60 patients examined. Twenty-five sentinel lymph nodes were negative, 23 of which were true negatives, while in two patients, other axillary lymph nodes were involved. In the other 35 patients, the sentinel lymph nodes were positive.

In the 54 patients, in whom both the blue dye and the probe were used together, the sentinel lymph node was found in 37 (68.5%) patients with the blue dye and in all patients with the probe. In 35

**Table 2.** Number of sentinel lymph nodes identified by the technique using <sup>99m</sup>Tc human colloidal albumin in 376 consecutively enrolled breast cancer patients with a clinically negative axilla\*

No. of lymph nodes	No. of patients (%)
1	249 (66.2)
2	97 (25.8)
3	24 (6.4)
4	1 (0.3)
None	5 (1)
Total	376 (99.7)

\*Identification rate  $371/376 \times 100 = 98.7\%$ .

patients, the blue node was the same as that which took up tracer. In two patients, a different lymph node took up blue dye.

### Intraoperative Pathologic Examination of Sentinel Lymph Node

Of the 192 patients examined intraoperatively by usual frozen section, 55 were positive and 137 were negative. All 55 patients with positive sentinel lymph nodes were confirmed by definitive histologic examination, while 26 sentinel lymph nodes negative on frozen section examination were found to contain microfoci of metastatic cells on definitive histologic examination. This gave a false-negative rate of 32.1% (26 of 81 patients; 95% CI = 22.2%–43.4%) that we considered too high to justify use of the sentinel lymph node finding to decide whether or not to perform complete axillary dissection. The new technique described in the "Pathology" section was therefore devised to provide a definitive histologic diagnosis of the lymph node intraoperatively and applied to the subsequent 119 patients. The procedure required 40–50 minutes, during which surgery on the breast was completed.

The sentinel lymph node was found in all 119 patients scheduled for the exhaustive intraoperative frozen section method. The findings were 67 negative sentinel lymph nodes, in three of which the axilla as a whole was positive (false-negative

rate = 5.5%; 95% CI = 1.1%–15.1%); the other 52 sentinel lymph nodes were positive.

### Axillary Involvement According to Primary Tumor Characteristics

We assessed the relation of axillary lymph node metastases to various characteristics of the primary carcinoma (Table 3). The univariate analysis showed that all variables except estrogen receptor status were significantly associated with nodal metastases. Grade 1 carcinomas had a low rate of axillary metastases, while there was no difference between grades 2 and 3 in terms of rate of axillary involvement. The final logistic model, which fitted the data well ( $\chi^2 = 6.65$ ; 7 df;  $P = .47$ ), showed that nodal involvement was associated with larger tumors, grades 2 and 3, and peritumoral vascular invasion, but not Ki67 or estrogen receptor status.

### Predictive Value of Sentinel Lymph Nodes

The sentinel lymph nodes were positive for metastases in 168 of the 371 assessable patients (Table 4). In 51 (30.3%) of these 168 patients, the involvement was limited to a single focus of micrometastasis (<0.2 cm). In 73 (43.5%), the sentinel lymph node was the only metastatic lymph node (24 of which were micrometastatic). Two hundred three patients

**Table 3.** Rates of axillary metastases in relation to pathologic and biologic variables in the 371 breast carcinomas\*

Variable	No. of patients	No. of patients with positive axillary lymph nodes	% (95% confidence interval)†
T <2 cm	257	112	43.6 (37.4–49.9)
T ≥2 cm	114	68	59.6 (50.1–68.7)
			$P = .006$
ER positive	285	138	48.4 (42.5–54.4)
ER negative	82	40	48.8 (37.6–60.2)
			$P = 1.000$
Ki-67 <20%	222	97	43.7 (37.1–50.5)
Ki-67 ≥20%	149	83	55.7 (47.3–63.8)
			$P = .031$
G1‡	69	18	26.1 (16.3–38.1)
G2	173	95	54.9 (47.2–62.5)
G3	118	63	53.4 (44.0–62.6)
			$P < .0001$
PVI negative	235	79	33.6 (27.6–40)
PVI positive	136	101	74.3 (66.1–81.4)
			$P < .0001$

\*Estrogen receptors (ERs) were evaluated in 367 patients, grading (G1, G2, and G3) was determined in 360 patients. Peritumoral vascular invasion (PVI), tumor size (T), and proliferative index (Ki67) were determined in all patients.

†The statistical test used is the chi-squared test.

‡In all cases, histologic grading was not available.

**Table 4.** Concordance between sentinel lymph node evaluation and definitive status of all axillary lymph nodes in 371 patients with breast cancer\*

Sentinel lymph node	Axillary lymph nodes†	No. of patients (% of total)
Positive	Positive	168 (45.3)
Negative	Negative	191 (51.5)
Negative	Positive	12 (3.2)
Total		371 (100)

\*Concordance 359/371 (96.8%).

†In 70 patients, the sentinel lymph node was the only positive lymph node.

$$\text{Sensitivity} = \frac{\text{Number of patients with a positive sentinel lymph node biopsy}}{\text{Number of patients with axillary lymph node metastases}} = \frac{168}{180} = 93.3\%$$

$$\text{Specificity} = \frac{\text{Number of patients with a negative sentinel lymph node biopsy}}{\text{Number of patients with no axillary lymph node metastases}} = \frac{191}{191} = 100\%$$

$$\text{Positive predictive value} = \frac{\text{Number of patients with axillary lymph node metastases}}{\text{Number of patients with a positive sentinel lymph node biopsy}} = \frac{168}{168} = 100\%$$

$$\text{Negative predictive value} = \frac{\text{Number of patients without axillary lymph node metastases}}{\text{Number of patients with a negative sentinel lymph node biopsy}} = \frac{191}{203} = 94.1\%$$

Overall accuracy =

$$\frac{\text{Number of patients with true-positive and true-negative sentinel lymph node biopsies}}{\text{Number of patients in whom sentinel lymph nodes were identified}} = \frac{359}{371} = 96.8\%$$

$$\text{False-negative rate} = \frac{\text{Number of patients with a negative sentinel lymph node biopsy}}{\text{Number of patients with axillary lymph node metastases}} = \frac{12}{180} = 6.7\%$$

(54.7%) had negative sentinel lymph nodes. In this group, the axillary lymph nodes were totally negative in 191 patients (94.1%), while 12 patients had metastases in other lymph nodes, mainly at the first level (nine patients); the first and second levels were involved in two patients, while in one patient, all three levels were involved. Of these 12 patients, eight had one positive lymph node, one had two positive lymph nodes, two had three positive lymph nodes, and one had four positive lymph nodes. The rate of positive other axillary lymph nodes among patients with negative sentinel lymph node was 5.9% (95% CI = 3.1%–10.1%). The overall concordance between sentinel lymph node and axillary lymph node status was 96.8% (359 of 371), while the rate of false negatives among patients with at least one positive lymph node was 6.7% (12 of 180; 95% CI = 3.5%–11.4%).

Forty-six patients had primary tumors with multicentricity or extensive multifocality (>3 cm between the extremes); 31 of them had involved axillary lymph nodes. Among these were three patients with negative sentinel lymph nodes (9.7%; 95% CI = 2.2%–28.2%). None of the variables we investigated (multicentricity, age, tumor size and site, proliferative index, and estrogen or progesterone receptor status) were significantly associated with falsely negative sentinel lymph node using Fisher's exact test.

## DISCUSSION

Our results show that radio-guided biopsy of the sentinel lymph node in breast cancer is an effective and useful procedure. The high rate of identified sentinel lymph nodes, the ease of probe-guided node dissection, the reliability of the new multi-level method of frozen section examination, the absence of risk of exposure to radioactivity by staff, and the acceptably low rate of false negatives (particularly in relation to the non-negligible rate of false negatives in complete axillary dissection) suggest that the procedure may represent an important step forward in the staging of the axilla in breast cancer. For surgeons who are experienced in axillary lymph node dissection, no special training in the technique is required, unlike the blue-dye method for which it is suggested that 30–50 dissections are required to reach full competence (9). Our staff includes seven senior surgeons, who are now involved full-time in breast and axillary surgery.

We were able to identify the sentinel lymph node in 371 of 376 patients, with an identification rate of 98.7%. Furthermore, the method correctly predicted the state of the axilla in a high percentage of patients (96.8%), although there were 12 patients in whom the sentinel lymph node was negative but other axillary lymph nodes were positive (3.2% of all patients).

The finding of 73 patients (43.5% of positive sentinel lymph nodes) (95% CI = 35.8%–51.3%) in whom the sentinel lymph node was the only positive axillary lymph node confirms the logical basis of the sentinel lymph node technique.

In a previous paper (14) that included findings from some of the patients described in this report, we noted the problem of the limited reliability of a negative finding on frozen-section examination, which was too often contradicted by the final histologic examination. This has now been solved by our new frozen section method, which examines some 30 sections of the sentinel lymph node with additional evaluation of the same number of sections immunohistochemically if hematoxylin–eosin staining is negative. Previous studies on large series of patients have confirmed the greater sensitivity of methods that, in addition to hematoxylin–eosin, also use immunocytochemical methods to reveal micrometastases. Thus, Giuliano (12) reported that 42.3% of negatives by hematoxylin–eosin were positive by use of anticytokeratin antibodies; similar figures were reported by Reintgen (27) and Krag et al. (28). We conclude from this that sentinel lymph node biopsy can probably improve axillary staging by revealing micrometastases; in such patients, adjuvant therapy (in addition to axillary dissection) can be planned if necessary.

We therefore believe that our new method of evaluating the sentinel lymph node provides a definitive histologic examination performed intraoperatively and can be used as a basis for deciding whether or not to perform total axillary clearance, without the risk that a second operation on the axilla will be necessary a few days later should standard paraffin sections prove micrometastatic.

Although we found no significant association between multifocality (primary carcinoma extending over >3 cm) and false negatives (perhaps because of the small number of patients and consequent low statistical power), we believe on biologic grounds that multifocality may reduce the ability of the sentinel lymph node to predict the state of the axilla, since multifocal tumors are likely to involve an extended area of the lymphatic network of the breast. We feel that patients with multifocality should not be candidates for sentinel lymph node biopsy.

Two decades ago, the treatment of

breast cancer was transformed by the demonstration that conservative surgery is as effective as mastectomy (2). Today we seem to be on the threshold of a development that may allow axillary dissection to be foregone in a large percentage of patients. Our data indicate that sentinel lymph node biopsy can reliably predict the state of the axilla, so that when this node is healthy, axillary dissection can be safely avoided. This is important since the removal of axillary lymph nodes deprives the patient of lymphatic tissue, which, if healthy, is best retained. Axillary dissection may have other sequelae, such as reduced arm motility, reduced skin sensitivity, pain, lymphoedema, and increased susceptibility to infections (29,30).

This development is particularly important in view of the changing presentation of primary breast cancer. Small tumors are found with ever-increasing frequency, and the percentage of patients with axillary metastatic involvement has declined greatly (31). Furthermore, important prognostic information can be obtained by investigation of the biologic properties of primary tumor (32-34).

Another possible clinical application of the sentinel lymph node technique would be to stage the axilla of patients candidate for neoadjuvant chemotherapy. It is important to know the pathologic condition of the axillary lymph nodes before preoperative chemotherapy because a negative finding afterward could be due either to prior lack of involvement or sterilization by the treatment (35).

An open problem is the case in which the sentinel lymph node is minimally involved (36,37). Our data show that sentinel lymph nodes involved by microfoci of cancer cells are, however, associated with a considerable rate of metastatic involvement in the remaining axillary lymph nodes (27 [53.0%] of 51; 95% CI = 38.5%-67.1%). Therefore, we believe that the presence of microfoci in the sentinel lymph node is an indication to perform a total axillary dissection.

In conclusion, our data indicate that sentinel lymph node biopsy can accurately stage the axilla, possibly allowing axillary dissection with all its unpleasant side effects, to be foregone in patients where the sentinel lymph node is clear. However, we believe that a clinical trial that randomizes patients to sentinel lymph node biopsy versus total axillary dissec-

tion is needed to demonstrate beyond all doubt that the method is efficacious.

Probe-guided biopsy is easy to apply, does not require special surgical training, and the whole procedure is associated with a low risk of false negatives. We anticipate that the technique will be widely adopted to stage the axilla in patients with breast cancer with clinically negative lymph nodes. We suggest, however, that the procedure is not suitable for patients with extensive primary multifocality.

We expect that large-scale implementation of the sentinel lymph node technique will reduce the cost of treatment as a result of shorter hospitalization times and will also reduce indirect costs because motor compromise and lymphoedema as a result of axillary emptying will become rare.

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## NOTES

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## Cyclin D1 Proteolysis: a Retinoid Chemoprevention Signal in Normal, Immortalized, and Transformed Human Bronchial Epithelial Cells

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**Background:** Retinoids (derivatives of vitamin A) are reported to reduce the occurrence of some second primary cancers, including aerodigestive tract tumors. In contrast,  $\beta$ -carotene does not reduce the occurrence of primary aerodigestive tract cancers. Mechanisms explaining these effective retinoid and ineffective carotenoid chemoprevention results are poorly defined. Recently, the all-*trans*-retinoic acid (RA)-induced proteolysis of cyclin D1 that leads to the arrest of cells in G<sub>1</sub> phase of the cell cycle was described in human bronchial epithelial cells and is a promising candidate for such a mechanism. In this study, we have investigated this proteolysis as a common signal used by carotenoids or receptor-selective and receptor-nonselective retinoids. **Methods:** We treated cultured normal human bronchial epithelial cells, immortalized human bronchial epithelial cells (BEAS-2B), and transformed human bronchial epithelial cells (BEAS-2B<sub>NNK</sub>) with receptor-selective or receptor-nonselective retinoids or with carotenoids and studied the effects on cell proliferation by means of tritiated thymidine incorporation and on cyclin D1 expression by means of immunoblot analysis. We also examined whether calpain inhibitor I, an inhibitor of the 26S proteasome degradation pathway, affected the decline (i.e., proteolysis) of cyclin D1. **Results:** Receptor-nonselective retinoids were superior to the carotenoids studied in mediating the decline in cyclin D1 expression and in suppressing the growth of bronchial epithelial cells. Retinoids that activated retinoic acid receptor  $\beta$  or retinoid X receptor pathways preferentially led to a decrease in the amount of cyclin D1 protein and a cor-

responding decline in growth. The retinoid-mediated degradation of cyclin D1 was blocked by cotreatment with calpain inhibitor I. **Conclusions:** Retinoid-dependent cyclin D1 proteolysis is a common chemoprevention signal in normal and neoplastic human bronchial epithelial cells. In contrast, carotenoids did not affect cyclin D1 expression. Thus, the degradation of cyclin D1 is a candidate intermediate marker for effective retinoid-mediated cancer chemoprevention in the aerodigestive tract. [*J Natl Cancer Inst* 1999;91:373-9]

Lung cancer is the leading cause of cancer mortality for men and women in the United States. Because curative therapy for disseminated non-small-cell lung cancer does not yet exist, effective chemoprevention of lung cancer in patients with prior tobacco exposure would have a major impact on reducing lung cancer mortality. Retinoids, natural and synthetic derivatives of vitamin A, have *in vitro* and *in vivo* chemoprevention activities. Clinical trials have shown that retinoids are active in treatment of some second cancers. Treatment with 13-*cis*-retinoic acid (13-*cis*-RA) has reduced second aerodigestive tract cancers in patients with previously resected head and neck cancers (1). Treatment with retinyl palmitate has reduced second primary lung cancers in patients who have had completely resected lung cancers (2). An acyclic retinoid, polyprenoic acid, inhibited development of second hepatocellular carcinomas in patients with resected or ablated

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