Sentinel node detection in pre-operative axillary staging

Giuseppe Trifirò[1], Giuseppe Viale[2], Oreste Gentilini[3], Laura Lavinia Travaini[1], Giovanni Paganelli[1]

[1] Division of Nuclear Medicine, European Institute of Oncology, Milan, Italy
[2] Division of Pathology, European Institute of Oncology, Milan, Italy
[3] Division of Senology, European Institute of Oncology, Milan, Italy

Published online: 22 April 2004

Abstract. The concept of sentinel lymph node biopsy in breast cancer surgery is based on the fact that the tumour drains in a logical way via the lymphatic system, from the first to upper levels. Since axillary node dissection does not improve the prognosis of patients with breast cancer, sentinel lymph node biopsy might replace complete axillary dissection for staging of the axilla in clinically N0 patients. Sentinel lymph node biopsy would represent a significant advantage as a minimally invasive procedure, considering that about 70% of patients are found to be free from metastatic disease, yet axillary node dissection can lead to significant morbidity. Subdermal or peritumoural injection of small aliquots (and very low activity) of radiotracer is preferred to intratumoural administration, and ^{99m}Tc-labelled colloids with most of the particles in the 100–200 nm size range would be ideal for radioguided sentinel node biopsy in breast cancer. The success rate of radioguidance in localising the sentinel lymph node in breast cancer surgery is about 97% in institutions where a high number of procedures are performed, and the success rate of lymphoscintigraphy in sentinel node detection is about 100%. The sentinel lymph node should be processed for intraoperative frozen section examination in its entirety, based on conventional histopathology and, when necessary, immune staining with anti-cytokeratin antibody. Nowadays, lymphoscintigraphy is a useful procedure in patients with different clinical evidence of breast cancer.

Keywords: Breast cancer – Sentinel lymph node – Radiocolloid – Lymphoscintigraphy – Sentinel lymph node biopsy

Eur J Nucl Med Mol Imaging (2004) 31 (Suppl. 1): S46–S55
DOI 10.1007/s00259-004-1526-9

Giovanni Paganelli (✉)
Division of Nuclear Medicine,
European Institute of Oncology, Milan, Italy
e-mail: direzione.mnu@ieo.it
Tel.: +39-02-57489043, Fax: +39-02-57489040

Introduction

Sentinel lymph node (SLN) localisation and biopsy represents one of the most important developments in surgery and has already produced important changes in the management of patients affected by early infiltrating breast carcinoma. Sentinel lymph node biopsy (SLNB) was first applied in melanoma patients by Morton and colleagues [1]; they intra-operatively injected the patent vital blue dye close to the primary lesion, and the blue-stained SLN was later found by dissection, following tracer diffusion.

Subsequently this technique was proposed as a method of disease staging in breast cancer patients [2] in order to permit the use of less aggressive surgical treatment that would not compromise quality of life. In fact, removal of axillary nodes in the presence of breast cancer is performed for staging and not with curative intent [3], and axillary dissection is burdened by a significant rate of immediate and delayed possible complications such as lymphoedema, paraesthesia, pain and restriction of arm motion. Nevertheless, information on axillary nodes is important in determining the appropriate type of adjuvant treatment, and SLNB has been proposed as an alternative to routine axillary clearance for the determination of nodal status.

The concept of the "sentinel lymph node" implies that lymphatic metastasis is not a random event but rather is based on an orderly and predictable pattern of lymph flow from the primary site to the regional lymph node basin. Sequential progression of tumour cells is assumed to occur, with the first lymph node (the SLN) filtering the afferent lymph and thereby entrapping the tumour cells. Both experimental evidence and clinical data support the hypothesis that there is an orderly and predictable pattern of lymphatic drainage from the breast to the regional lymph nodes and progressive involvement of the axillary lymph nodes.

It is to be noted that when mammography is used within the context of a screening programme, breast carcinomas are diagnosed at earlier stages, when the rate of node-positive disease is low. Moreover, lymphatic

spread within the axilla generally proceeds from the first Berg level to the third, and skip metastases are infrequent [4, 5].

Several studies have demonstrated that lymphoscintigraphy in combination with gamma probe-guided surgery is the best procedure to identify and remove the SLN in breast cancer patients [6], being more suitable and sensitive than blue dye mapping. The method of lymphoscintigraphy to be used for SLN localisation is still controversial. We have already described a reliable lymphoscintigraphic technique in previous works [7, 8].

Lymphoscintigraphy

In recent years, hundreds of studies have been published on lymphoscintigraphy in breast cancer, and the reported experience and data have often been discordant. The main areas of controversy concern radiopharmaceuticals, the site of injection and mode of administration, the optimal activity and the appropriate radiotracer volume.

Three types of radiopharmaceutical preparations are commonly employed for lymphoscintigraphy: ^{99m}Tc-sulphur colloid is the most commonly used in the United States, either unfiltered (size about 15–5,000 nm) or filtered. Some authors [9, 10] still claim the superiority of the unfiltered over the filtered preparation [11]. In Europe, ^{99m}Tc-nanocolloid is more frequently used, with particles between 4 and about 100 nm (95% of the particles are <80 nm). Finally, ^{99m}Tc-antimony trisulphide (3–30 nm) is widely employed for SLN procedures [12] in Australia and Canada. It is generally considered that radiocolloid with most of the particles ranging between 100 and 200 nm in size represents the best compromise between fast and efficient lymphatic drainage and satisfactory retention in the SLN [13].

The site of injection is the most crucial parameter, and heavily affects the final choice of the other two main parameters, volume and activity. Intratumoural injection [14] represents a natural extension of the technique developed earlier with vital blue dye: it is generally characterised by a relatively large volume of radiotracer (at least 4 ml) and a relatively high injected activity of radiocolloid (37–370 MBq). Interstitial administration can be performed using peritumoural/intraparenchymal injection and subdermal/intradermal injection [15] (Fig. 1). The principle of intraparenchymal administration is to inject the tracer in a site immediately adjacent to the tumour, that is, in the space with a supposedly normal lymphatic system, which is the only possible drainage pathway for fluids, particles and cells leaving the tumour via the extravascular route. Finally, peri-areolar/subareolar tracer injection [16] is based on the existence of a lymphatic plexus around each lobule of the mammary gland that follows the path of the galactophore ducts, converging to the areola to form the Sappey subareolar plexus, which is part of the general subcutaneous plexus [17]. It is in fact reasonable to assume that these various techniques are complementary [18].

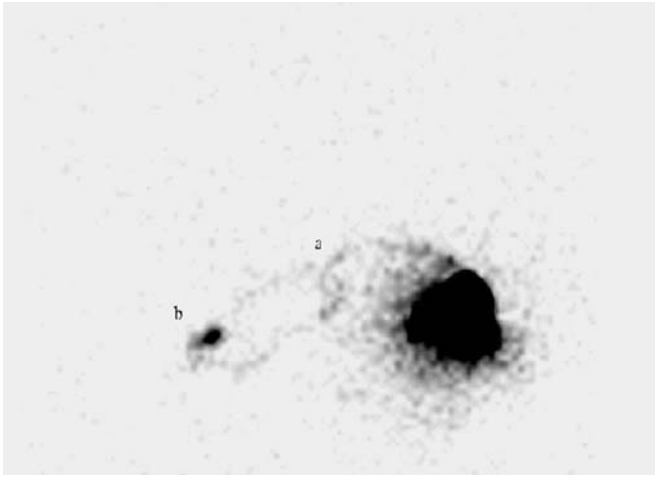

Fig. 1. Subdermal injection, right breast: standard method used at the European Institute of Oncology. Note the lymphatic vessels (*a*) and only one lymph node (*b*) at the right axilla

Several technical aspects of lymphoscintigraphy have been optimised by our group, based on detailed investigations [8, 19, 20, 21]. In a large series of patients we performed a comparative study using antimony sulphide colloids with a particle size of <50 nm, colloidal particles of HSA sized <80 nm and colloidal particles of HSA sized 0.2–1 μm, administered either subdermally or peritumourally in a volume ranging from 0.2 to 3 ml and with very low activity [8]. The results suggested the use of larger colloidal particles (0.2–1 μm) to be most appropriate because while only one or two SLNs were identified, smaller colloids were often trapped in several nodes and retained in the lymphatic channels, which would cause the surgeon problems in distinguishing between the true SLN and other radioactive sources [7].

The likelihood of visualising a lymphatic duct and a draining lymph node increases when the radiocolloid is injected in the skin overlying the mammary gland, because lymphatic drainage from the skin is richer and faster than drainage from the remaining breast parenchyma [22]. Axillary SLNs can therefore be efficiently visualised as early as 20–30 min after intradermal injection of radiocolloid, thus making the entire lymphoscintigraphic procedure highly practicable. Nevertheless, convenient timing is not the only factor that makes the intradermal administration route such an attractive option for SLNB in early breast cancer. Reliability of this approach for SLN identification has a sound anatomical and physiological basis. With this administration approach, a small volume of tracer (between 0.2–0.3 ml), containing 10–15 MBq of ^{99m}Tc-colloid, is injected as a single aliquot in the skin directly overlying the tumour. Based on how deeply the injection is performed, tracer administration is defined as intradermal when the needle is almost

tangential to the skin surface and a classical urticarial wheal ensues, and as subdermal when injection is a little deeper (this is signalled by reduced resistance to penetration of the needle) and the wheal is less obvious. Clearly, there is some overlap between these two modalities and the two terms are often used interchangeably, also because of the wide variation in the thickness of the skin overlying the breast as a function of individual characteristics, breast size and age of the patient.

Some investigators perform peri-areolar tracer injection (usually with the injection of four aliquots) as a modification of the subdermal route. However sound its pathophysiological basis may be (due to the rich connections of the subareolar plexus with the general subcutaneous plexus), we do not favour this technique, in part because it causes some discomfort to patients. This approach may also demonstrate sites of drainage of the breast per se as opposed to specific drainage of the tumour.

Advantages of the intradermal-subdermal injection technique are its practicability (it requires a minimum of training), the administration of a small volume using a single injection, the fast visualisation of lymphatic drainage pathways and the low administered activity, which results in fewer technical problems during lymphoscintigraphic imaging and intraoperative gamma probe counting. Some studies have compared the lymphoscintigraphic pattern and the performance in respect of SLN identification when using both the intradermal and the peritumoural approach in the same patients [16, 23, 24, 25]. Although the two techniques are reported to yield virtually equivalent results in the vast majority of patients [16, 26, 27], some authors have reported a sizeable proportion of discordant results concerning SLNs in the axilla and/or in the internal mammary chain (IMC) [25, 28].

Whatever method is used, we believe that, with rare exceptions, lymphoscintigraphy must be able to localise axillary SLNs.

Internal mammary chain

The internal mammary lymphatic trunks originate from the anterior pre-pericardial lymph nodes lying upon the upper surface of the diaphragm. These nodes receive collecting lymphatics from the anterior and superior portion of the liver via the falciform ligament, from the anterior portion of the diaphragm, and from the upper portion of the rectus abdominis muscle, as well as from the lower inner sector of the adjacent mammary gland [29]. The main efferent lymphatics from the breast to the internal mammary route emerge from the deep portion and from the medial edge of the mammary gland.

When lymphoscintigraphy is performed after subdermal/intradermal radiocolloid injection, the detection of SLNs outside the axilla is an unlikely event, occurring in 1–2% of cases [30, 31]. We performed a pilot study in order to establish whether a deep injection can visualise the IMC nodes in a high percentage of cases, according to our standard lymphoscintigraphic protocol (Fig. 2). Three points emerged from this study as regards IMC lymphoscintigraphy and localisation:

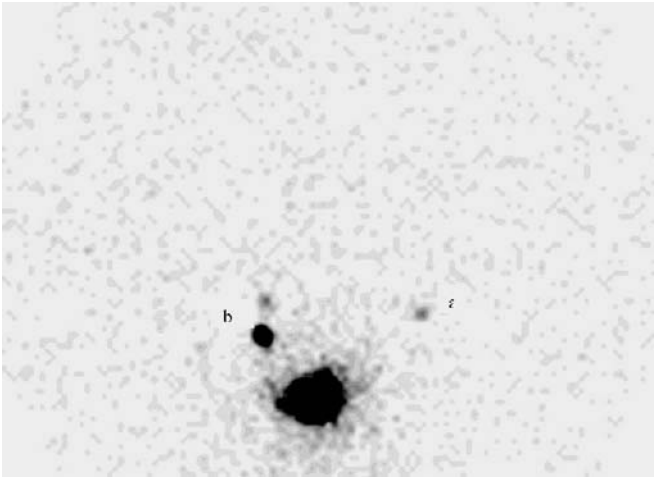

Fig. 2. Deep injection, inner quadrant, left breast. Please note the lymph nodes' double drainage at the axilla (*a*) and IMC (*b*)

- A deep injection (under the tumour) is a suitable way of administering ^{99m}Tc-colloids to visualise IMC nodes.
- Uptake by the IMC nodes occurred in 65% of lesions located in the inner quadrant.
- An involved SLN in the IMC was found in 8.5% of the evaluated cases. According to the UICC staging classification, these cases "migrated" from N0 (two cases) and N1 (three cases) to N3. Without internal mammary sampling, these patients would have been understaged [19].

Randomised trials

Six prospective randomised trials have been designed to validate SLNB. Actually, many centres and surgeons adopted SLNB as standard practice in the treatment of early breast cancer before the publication of these trials. This approach has been accepted because it has been clearly demonstrated that axillary clearance is performed not with curative intent but only for informative purposes [3].

The results of the first prospective randomised study have recently been published by Veronesi and co-workers [32]. Five hundred and sixteen women with primary breast cancer ≤2 cm in size were randomly assigned to undergo either SLNB and simultaneous axillary dissection (AD) or SLNB followed by AD only in the event of a positive SLNB. The primary endpoint of the study was

the predictive power of the status of the SLN, measured in terms of the percentage of cases of axillary involvement detected by SLNB in relation to the percentage found by routine AD. A SLN was positive in 32.3% in the AD group and in 35.5% in the SLN group. In the AD group, the overall accuracy, the sensitivity and the specificity of the SLN was 96.9%, 91.2% and 100% respectively. The false negative rate of 8.8% and the negative predictive value of 95.4% were consistent with previous studies [6, 33, 34]. There was less pain and better arm mobility in patients who underwent SLNB only than in those who also underwent AD. After a median follow-up of 46 months, disease-free and overall survival rates were not significantly different between the two groups. In particular, overt axillary metastases were not detected in patients who had undergone SLNB only.

In the U.S. two studies have been launched [35, 36]. The National Surgical Adjuvant Breast and Bowel Project (NSABP) trial B-32 [37] has been designed in order to evaluate whether SLNB alone is equivalent to AD in terms of long-term control of regional disease, disease-free survival and overall survival, as primary end-points, with accrual of 5,500 patients with clinically negative axillae and a pathologically negative SLN. The American College of Surgeons–Oncology Group (ASOCOG) study (protocol Z0011) has now stopped accrual for a trial evaluating survival in women with pathologically positive SLNs who are randomly assigned to either AD or observation alone [38, 39].

In Europe three additional trials are ongoing. The European Organisation on Research and Treatment of Cancer (EORTC) is enrolling women in the AMAROS (After Mapping of the Axilla: Radiotherapy or Surgery?) trial comparing complete AD with axillary radiotherapy in women with positive SLNs; the target is accrual of 3,485 patients within 3 years [40].

The Medical Research Council in the United Kingdom has funded the Axillary Lymphatic Mapping Against Nodal Axillary Clearance (ALMANAC) trial, where around 1,300 women with clinically negative axillary nodes are expected to be randomised either to conventional surgery or to SLNB. The primary endpoints are axillary morbidity, health economics and quality of life following SLNB as compared to conventional axillary procedures [41].

Finally, the clinical and prognostic value of SLN micrometastases is being evaluated in the International Breast Cancer Study Group (IBCSG) trial 23.01, in which about 1,960 women with a diagnosis of micrometastases or isolated tumour cells only in the SLN are being randomly assigned to either complete AD or no further axillary treatment.

Sentinel lymph node biopsy: when and where?

In the proceedings of the Consensus Conference (Philadelphia, 2001) on the role of SLNB in carcinoma of the breast, the panel considered SLNB to be a suitable replacement for AD in carcinoma with a diameter below 3 cm and no clinically suspicious palpable axillary nodes [42].

The rapid spread of SLNB has led to its use in clinical settings previously considered contraindications to SLNB. It has to be clearly stated, however, that all the settings discussed below are at least controversial indications for SLNB, and that in these cases SLNB should be limited to centres with extensive experience in this procedure, and should only be used after careful discussion of the available data with the patient.

SLNB after primary chemotherapy

Former papers on SLNB after primary chemotherapy (PC) reported that the false negative rate of SLNB was higher in patients who had received PC than in those who had not [43, 44, 45, 46]. More recent and larger studies have demonstrated that, with increasing experience, the identification and false-negative rates of SLNB are similar to those reported in the absence of PC [47, 48, 49, 50]. Therefore, in women with a clinically negative axilla before the start of PC, SLNB might be considered after the completion of medical treatment if no progression has occurred. In patients with suspicious axillary nodes at presentation which have been "downstaged" to N0 by medical treatment, SLNB might also be considered an option in the hands of surgeons with extensive experience in this procedure. SLNB is obviously not recommended for patients whose axillary nodes remain clinically suspicious after PC.

Some considerations might be added regarding the opportunity to gain pathological information on the nodal status before starting preoperative treatment. Axillary status before PC provides prognostic information that could be missed following PC, and therefore it would be wise to perform axillary staging before PC in the event of a clinically negative axilla. Afterwards, if the node(s) were negative, axillary dissection following PC could be avoided, whereas in the case of a positive SLN, AD would be part of the surgical plan after medical treatment. This approach might also overcome the concern regarding the debated lower identification rate and sensitivity of SLNB after PC [51].

On the other hand, it is conceivable that the prognostic value of axillary staging following PC is even higher, since it already mirrors response to treatment. Moreover, performing SLNB before PC would lead to completion AD in all patients with positive SLNs, and therefore to the use of AD in a higher fraction of patients, given that PC may "sterilise" axillary node metastases in about 20% of patients [52].

Multicentric/multifocal breast cancer

Multifocal disease seems to be associated with a higher rate of nodal involvement than unifocal lesions of similar size [53]. Multiple foci of carcinoma, particularly when located in different quadrants of the breast, have been considered a relative contraindication to SLNB because of concerns that these tumours might involve more than one dominant lymphatic trunk draining to axillary nodes, and thus lead to an increased false-negative rate [54, 55]. In our initial experience with 163 women, two of the four patients with false-negative SLNB had multifocal disease [6]. Ozmen et al. [56], in a study conducted on 111 patients, 21 of whom had multifocal tumours, reported an accuracy of 93.7% with a false negative rate of 11.3% in the whole cohort of patients. Multifocality and tumour size (>2 cm) were significantly associated with decreased accuracy and increased false negative rates. Klimberg et al., however, reported that the rate of identification of the SLN was equal following either subareolar or peritumoural injection [16], and therefore multicentric cancers might drain first to the subareolar area and then to the SLN in the axilla, through a network of lymphatic vessels connecting different quadrants with the subareolar area [57]. Schrenk and Wayand [58] performed SLNB with either blue dye alone or blue dye and radiolabelled colloid injected in the subareolar area in 21 women with multicentric breast carcinoma who were prospectively evaluated and candidates for standard AD. The authors found a 100% identification rate of SLNs and no false negative SLNBs.

Two papers from the Memorial Sloan-Kettering Cancer Center have addressed the issue of SLNB in multicentric breast cancer. In the first [59], five patients with two tumours in distinct quadrants were injected at one site with technetium-labelled sulphur colloid and at the second site with isosulphan blue dye. Having identified at least one node that was both hot and blue within the axilla in all cases, the authors suggested that the lymphatic drainage of the entire breast coincides with the drainage of the tumour bed, regardless of its location. In the second paper, Tousimis et al. [60] considered 70 patients with multicentric/multifocal breast carcinoma who were submitted to mastectomy and SLNB with planned AD. The accuracy of SLNB was 96%, and the sensitivity, 92% (false negative rate 8%); these results are comparable to those of published studies carried out in women presenting with unifocal disease. All three patients with inaccurate SLNB had dominant invasive tumour larger than 5 cm, and in one case axillary disease was palpable at surgery.

In the study by Kumar et al. [61] on 48 patients with multicentric/multifocal breast carcinoma, success rate, sensitivity, negative predictive value and accuracy were 93%, 100%, 100% and 100% using the radiocolloid probe, 87%,100%, 100% and 100% using blue dye, and 93.5%, 100%, 100% and 100% using combined methods. The authors concluded that SLN localisation maintained a high negative predictive value in multicentric/multifocal breast cancer, contrary to the common belief that a higher rate of false negative results occurs in this subset of patients, and they proposed its routine use instead of AD in these patients, too.

Previous breast biopsy

Some authors have suggested that altered lymphatic drainage decreases the likelihood of successful lymphatic mapping and, indeed, that SLNB for breast cancer may be less accurate after excisional biopsy of the primary tumour [22, 62, 63]. Other authors [64, 65] claim that neither biopsy type nor type of definitive surgical procedure significantly affects the accuracy of SLN biopsy for breast cancer, and that SLNB can be performed accurately after excisional biopsy and is equally effective in patients undergoing partial mastectomy or total mastectomy.

Ductal carcinoma in situ and SLNB

In the presence of ductal carcinoma in situ (DCIS), AD is not indicated since this is an intra-epithelial neoplasm which typically does not have the potential for metastasis. The prevalence of axillary metastases, which is lower than 2% [66], does not justify the significant morbidity associated with lymph node removal. The techniques of lymphatic mapping and SLNB, however, have also been applied to patients with DCIS, resulting in some series in an unexpectedly high rate of detection of metastases. Pendas et al. [67] reported a 5.7% rate in 87 patients, though after exclusion of micro-invasion this rate decreased to 4.6%. Of the 76 patients with high-risk DCIS described by Klauber-DeMore et al. [68], 12% had metastases in the SLN, but further evaluation allowed exclusion of patients with invasion and the actual rate was lowered to 6.6%. In the paper by Cox et al. [69], a 13% rate of positive SLNs was found in 195 patients with pure DCIS, but lack of data on the extent of sampling makes comparison difficult.

In our experience in 223 patients with pure DCIS [70], despite very extensive examination of the SLNs, the percentage of SLN metastases remained low (3.1%) and consistent with the literature following complete AD. We did not find any biological features to be associated with an increased risk of positive SLNB. Furthermore, of the seven patients with metastatic SLNs, six had undergone a previous breast biopsy, whereas only 36.1% of the patients with negative SLNs underwent a diagnostic invasive procedure, either surgical biopsy or a vacuum-assisted biopsy. The chance of passive transport of dislocated epithelial cells to the SLN following an invasive preoperative procedure has already been hypothesised [71].

From a practical point of view, SLNB is not routinely necessary in women with a diagnosis of pure DCIS but it can be discussed with the patient in order to avoid a subsequent operation if micro-invasive cancer is found at the final histology. We recommend SLNB whenever a mastectomy with immediate reconstruction is required. We also recommend it in patients with a cluster of microcalcifications when there is a reasonably high associated rate of micro-invasion at the final histology (e.g. cluster >1 cm or incomplete removal of the entire cluster).

Male breast cancer

Breast carcinoma is a rare disease in males, accounting 1% of the malignancies of breast and less than 1% of all tumours in men. It is estimated that in 2001 in the United States about 1,500 men developed breast cancer and that 400 of them died in the same year due to the disease. The life-time risk of having breast cancer is calculated to be 0.11% in men versus 13% in women [72].

In men, breast carcinoma has a poor prognosis, due to both its aggressiveness and delayed diagnosis [73]. As in women, axillary lymph node involvement represents the most important prognostic factor, and it is reported to be found in 50% of the cases in men [74].

Mastectomy according to Patey, or conservative surgery in combination with AD, represents the standard treatment of the disease. The risk factors and morbidity associated with AD are consistent and, as in women, include duration of surgery, serious arm lymphoedema and functional impotence.

Lymphoscintigraphy and SLNB are applied with the same modalities in males with breast carcinoma as in women [20, 75, 76], with similar advantages for the patient.

Pregnancy

In a recent review on breast cancer during pregnancy, it has been stated that AD is preferred because nodal metastases are commonly found, nodal status affects the choice of adjuvant chemotherapy and SLNB poses an unknown risk to the fetus from the radioisotope [77]. Patients with breast cancer during pregnancy are excluded from randomised studies on SLNB currently ongoing in the United States, and in the Consensus Conference on the role of SLNB in breast carcinoma the panel advised against SLNB in pregnant women until more data are available [40].

Nicklas and Baker [78] suggested that lymphoscintigraphy can be safely performed in pregnancy, with negligible risks for the fetus. In fact, the radioisotope remains trapped at the injection site or within the lymphatics, and the exposure to the fetus is essentially zero. Morita et al. [79] added that pregnancy per se should not be a contraindication to studying patients with lymphoscintigraphy.

In our opinion some practical recommendations might be given in order to further minimise the exposure of the fetus, such as avoiding contact with other patients who are potential sources of radioactivity (e.g. by scheduling a pregnant patient as the first procedure of the day, and keeping the patient in a single-bed room) and decreasing the time interval between lymphoscintigraphy and surgery, which might permit a reduction in the administered activity. Thus in pregnant patients SLNB could be performed within 2–3 h post injection of 3–5 MBq of ^{99m}Tc radiocolloids.

Local anaesthesia

SLNB might also be performed under local anaesthesia with sedation (e.g. using midazolam 2 mg i.v.), with excellent patient compliance and tolerability [21, 80].

Pathology

The histopathological examination of each SLN must be particularly accurate, to avoid a false negative or a false positive diagnosis. We have devised a very accurate protocol for the examination of axillary SLNs, which can be applied either to frozen sections for an intraoperative diagnosis or to fixed and embedded SLNs [81]. Upon receipt of the SLN, the pathologist must remove the perinodal fibrous-fatty tissue, and bisect the lymph node along the major axis. Small lymph nodes (up to 5 mm thick) may be processed uncut, while larger lymph nodes are sliced in 3- to 4-mm-thick slices.

From each moiety or slice of the SLN, 15 pairs of sections are cut at 50-mm intervals; whenever lymph node tissue is left, additional pairs of sections, cut at 100-mm intervals, are obtained until the node has been completely sectioned. One section from each pair is stained with haematoxylin and eosin (H&E) and examined. The mirror section is kept for possible immunohistochemical staining to ascertain the nature of any atypical cells, suspicious for malignancy, that are detected in the H&E preparations. We perform a rapid assay [82], based on a single incubation step with a monoclonal antibody to cytokeratins (MNF116) directly coupled to peroxidase via a polymer (EPOS Dako, Glostrup, Denmark). We normally avoid performing a traditional intraoperative examination of the SLNs, whereby only one to three frozen sections are examined and the rest of the node is left for the subsequent examination of permanent sections. In this event, a high rate of false negative intraoperative diagnoses is to be expected, with the need for a second operation to achieve complete AD in almost 17% of patients.

The above-described extensive histopathological examination of the SLN has been designed to identify in

the SLNs even micrometastatic disease (i.e. tumour foci up to 2 mm in size) or isolated tumour cells (ITCs), which can escape detection by less accurate evaluation. ITCs are defined as single neoplastic cells or small clusters of cells not larger than 0.2 mm which do not typically show evidence of metastatic activity (e.g. proliferation or stromal reaction) or penetration of vascular or lymphatic sinus walls [83]. These cells are more commonly identified with the use of immunohistochemical reactions, but may also be recognised in routinely stained sections. The actual prognostic value of axillary lymph node micrometastases is still the subject of debate, though most recent findings indicate that they correlate with a worse overall survival [84, 85, 86]. From a practical point of view, however, the first question to be addressed is whether the detection of micrometastases in the SLN should dictate completion of AD, or whether the risk of additional metastases to non-sentinel lymph nodes is low enough to allow axillary clearance to be spared, as has been suggested [87, 88]. We [89] and others [90] have demonstrated that the risk of additional metastases to non-sentinel axillary lymph nodes in the presence of micrometastatic disease in the SLN actually ranges from 22% to 25%, and that when ITCs are present in the SLN, patients have no less than a 15% risk of additional metastases. Accordingly, outside of randomised clinical trials designed to test the value of AD in these patients, the current practice in our institute is to complete AD in all patients with SLN micrometastases or ITCs. A randomised clinical trial has recently been launched by the International Breast Cancer Study Group (IBCSG) to assess whether patients with micrometastatic SLNs should necessarily undergo complete AD for a more extensive evaluation of the lymph node status, or whether the information obtained by examination of the SLN only, coupled with the morphological and biological characteristics of the primary tumour, is sufficient to plan proper adjuvant treatment.

More recently, molecular biology assays have also been adopted for the identification of so-called occult metastases, i.e. metastases not detected by morphological methods, including immunohistochemistry [91, 92, 93, 94]. Micrometastases in axillary lymph nodes detected by RT-PCR have been reported to be clinically significant, being an independent predictor of survival in a retrospective series of patients [89]. Some researchers have indicated that detection of tumour mRNA markers in the SLNs of breast carcinoma patients may be more accurate than histological examination in predicting axillary lymph node status, or have recommended reverse transcription-polymerase chain reaction (RT-PCR) as a more feasible and practical assay than extensive histological examination for an accurate diagnosis of SLN metastases, with a similar detection sensitivity [90, 91, 92].

We have compared the results of RT-PCR assays with those obtained by our extensive histopathological examination of the SLN from 123 patients [95]. A multiple-marker RT-PCR assay of five different tumour mRNA markers (cytokeratin 19, CEA, maspin, mammaglobin and MUC-1) showed a good sensitivity (95.6%) but a poor specificity (66.3%) when compared with histology, and a lower predictive value with respect to the status of the remaining non-sentinel axillary lymph nodes. In particular, we observed a high prevalence of positive RT-PCR assays in histologically uninvolved SLNs, possibly due to the occurrence of low-level expression of genes by illegitimate transcription in normal resident cells of the SLN. To overcome this limitation, we are currently exploiting the use of quantitative real-time RT-PCR assays for differentiating the low-level "illegitimate" transcription of some mRNA markers by non-neoplastic tissues from the expected higher expression in neoplastic cells.

Conclusion

It should be considered that introduction of the SLNB procedure in any given institution requires a combined effort which involves at least three different specialties, nuclear medicine, surgical oncology and pathology, with the addition of health physics.

However experienced a specialist is, there will be a definite, significant learning curve which depends on how rapidly the different operators develop the attitude that they are working as a single team. Thus, the learning curve should be related to the team rather than to the individual specialists, who will have to gain confidence in all the various steps of the procedure and at the same time rely on each other's contribution to the entire process.

Finally, SLN localisation has great potential to benefit patients; however, care must be taken to ensure fair and accurate information.

References

1. Morton DL, Wen DR, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg 1992; 127:392–399.
2. Giuliano AE, Jones RC, Brennan M, Statman RR. Sentinel lymphadenectomy and breast cancer. J Clin Oncol 1997; 15: 2345–2350.
3. Fisher B, Jeong JH, Anderson S, Bryant J, Fisher ER, Wolmark N. Twenty-five-year follow up of a randomized trial comparing radical mastectomy, total mastectomy, and total mastectomy followed by irradiation. N Engl J Med 2002; 22:567–575.
4. Veronesi U, Luini A, Galimberti V, Marchini S, Sacchini V, Rilke F. Extent of axillary involvement in 1446 cases of breast cancer. Eur J Surg Oncol 1990; 16:127–133.
5. Zurrida S, Morabito A, Galimberti V, et al. Importance of the level of axillary involvement in relation to traditional variables in the prognosis of breast cancer. Int J Oncol 1999; 15:475–480.

6. Veronesi U, Paganelli G, Galimberti V, et al. Sentinel-node biopsy to avoid AD in breast cancer with clinically negative lymph-nodes. Lancet 1997; 349:1864–1867.
7. Paganelli G, De Cicco C, Cremonesi M, et al. Optimized sentinel node scintigraphy in breast cancer. Q J Nucl Med 1998; 42:49–53.
8. De Cicco C, Cremonesi M, Luini A, Bartolomei M, Grana C, Prisco G, Galimberti V, Calza P, Viale G, Veronesi U, Paganelli G. Lymphoscintigraphy and radioguided biopsy of the sentinel axillary node in breast cancer. J Nucl Med 1998; 39:2080–2084.
9. Hill AD, Tran KN, Yeung H, Yeh SD, Borgen PI, Cody HS 3rd. Sentinel lymph node biopsy in breast cancer: unfiltered radioisotope is superior to filtered. J Am Coll Surg 1999; 188:377–381.
10. Cody HS 3rd, Borgen PI. State-of-the-art approaches to sentinel node biopsy for breast cancer: study design, patient selection, technique, and quality control at Memorial Sloan-Kettering Cancer Center. Surg Oncol 1999; 8:85–91.
11. Krag DN, Weaver DL, Alex JC, Fairbank JT. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma-probe. Surg Oncol 1993; 2:335–340.
12. Wilhelm AJ, Mijnhout GS, Franssen EJF. Radiopharmaceuticals in sentinel lymph-node detection—an overview. Eur J Nucl Med 1999; 26 (Suppl):S36–S42.
13. Mariani G, Moresco L, Viale G, Villa G, Bagnasco M, Canavese G, Buscombe J, Strauss HW, Paganelli G. Radioguided sentinel lymph node biopsy in breast cancer surgery. J Nucl Med 2001; 42:1198–1215.
14. Tanis PJ, Valdes Olmos RA, Muller SH, Nieweg OE. Lymphatic mapping in patients with breast carcinoma: reproducibility and lymphoscintigraphic results. Radiology 2003; 228:313–315.
15. Roumen RM, Geuskens LM, Valkenburg JG. In search of the true sentinel node by different injection techniques in breast cancer patients. Eur J Surg Oncol 1999; 25:347–351.
16. Klimberg VS, Rubio IT, Henry R, Cowan C, Colvert M, Korourian S. Subareolar versus peritumoural injection for location of the sentinel lymph node. Ann Surg 1999; 229:860–864.
17. Spratt JS, Schieber W, Dillard B. Anatomy and surgical technique of groin dissection. St. Louis: CV Mosby, 1965.
18. Alazraki NP, Styblo T, Grant SF, Cohen C, Larsen T, Aarsvold JN. Sentinel node staging of early breast cancer using lymphoscintigraphy and the intraoperative gamma-detecting probe. Semin Nucl Med 2000; 30:56–64.
19. Paganelli G, Galimberti V, Trifirò G, Travaini L, De Cicco C, Mazzarol G, Intra M, Rocca P, Prisco G, Veronesi U. Internal mammary node lymphoscintigraphy and biopsy in breast cancer. Q J Nucl Med 2002; 46:138–144.
20. De Cicco C, Baio SM, Veronesi P, Trifirò G, Ciprian A, Prisco G, Vento A, Rososchansky J, Viale G, Paganelli G. Sentinel node biopsy in male breast cancer. Nucl Med Commun 2004:in press.
21. Luini A, Gatti G, Frasson A, et al. Sentinel lymph node biopsy performed with local anesthesia in patients with early-stage breast carcinoma. Arch Surg 2002; 137:1157–1160.
22. Borgstein PJ, Pijpers R, Comans EF, et al. Sentinel lymph node biopsy in breast cancer: guidelines and pitfalls of lymphoscintigraphy and gamma probe detection. J Am Coll Surg 1998; 186:275–283.
23. Borgstein PJ, Meijr S, Pijpers R. Intradermal blue dye to identify sentinel lymph node in breast cancer. Lancet 1997; 349:1668–1669.
24. Borgstein PJ, Meijer S, Pijpers RJ, van Diest PJ. Functional lymphatic anatomy in breast cancer: echoes from the past and the periareolar blue method. Ann Surg 2000; 232:81–89.
25. Roumen RM, Geuskens LM, Valkenburg JG. In search of the true sentinel node by different injection techniques in breast cancer patients. Eur J Surg Oncol 1999; 25:347–351.
26. Borgstein P, Meijier S. Historical perspective of lymphatic tumour spread and the emergence of the sentinel node concept. Eur J Surg Oncol 1998; 24:85–89.
27. Keshtgar MRS, Ell PJ. Sentinel lymph node detection and imaging. Eur J Nucl Med 1999; 26:57–67.
28. Morton DL, Chan AD. The concept of sentinel node localization: how it started. Semin Nucl Med 2000; 30:4–10.
29. Handley RS, Trackray AC. Invasion of internal mammary lymph nodes in carcinoma of the breast. Br Med J 1954; 1:61–63.
30. Noguchi M, Tsugawa K, Miwa K. Internal mammary chain sentinel lymph node identification in breast cancer patient. J Surg Oncol 2000; 73:75–80.
31. Cox CE, Bass SS, Reintgen DS. Techniques for lymphatic mapping in breast carcinoma. Surg Oncol Clin North Am 1999; 8:447–468.
32. Veronesi U, Paganelli G, Viale G, et al. A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. N Engl J Med 2003; 349:546–553.
33. Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Ann Surg 1994; 220:391–401.
34. Krag DN, Weaver D, Ashikaga T, et al. The sentinel node in breast cancer—a multicenter validation study. N Engl J Med 1998; 339:941–946.
35. Wilke LG, Giuliano AE. Sentinel lymph node biopsy in patients with early-stage breast cancer: status of the National Clinical Trials. Surg Clin North Am 2003; 83:901–910.
36. Ross MI. Sentinel lymph node dissection in early-stage breast cancer: ongoing prospective randomized trials in the USA. Ann Surg Oncol 2001; 8:778–818.
37. Krag DN, Julian T. Expert perspectives: update on NSABP B-32, a randomized phase III clinical trial to compare sentinel node resection to conventional axillary dissection for clinically node-negative breast cancer. Breast Dis Year Book Q 2002; 13:113–114.
38. Grube BJ, Giuliano AE. Observation of the breast cancer patient with a tumor-positive sentinel node: implications of the ASOCOG Z0011 trial. Semin Surg Oncol 2001; 20:230–237.
39. Breast Study announcements. Durham, N.C.: American College of Surgeons Oncology Group, 2003. (Accessed July 18, 2003 at www.asocog.org/studies/organ_site/breast/.)
40. Hurkmans CW, Borger JH, Rutgers EJ, et al. Quality assurance of axillary radiotherapy in the EORTC AMAROS trial 10981/22023. Radiother Oncol 2003; 68:233–240.
41. Clarke D, Khonji NI, Mansel ER. Sentinel node biopsy in breats cancer: ALMANAC trial. World J Surg 2001; 25:819–822.
42. Schwartz GF, Giuliano AE, Veronesi U. Consensus Conference Committee. Proceedings of the consensus conference on the role of sentinel lymph node biopsy in carcinoma of the breast, April 19–22, 2001, Philadelphia, Pennsylvania. Cancer 2002; May 15; 94:2542–2551.
43. Bedrosian I, Reynolds C, Mick R, et al. Accuracy of sentinel lymph node biopsy in patients with large primary breast tumors. Cancer 2000; 88:2540–2545.

44. Cohen LF, Breslin TM, Kuerer HM, Ross MI, Hunt KK, Sahin AA. Identification and evaluation of axillary sentinel lymph nodes in patients with breast carcinoma treated with neoadjuvant chemotherapy. Am J Surg Pathol 2000; 24:1266–1272.
45. Nason KS, Anderson BO, Byrd DR, et al. Increased false negative sentinel node biopsy rates after preoperative chemotherapy for invasive breast carcinoma. Cancer 2000; 89:2187–2194.
46. Brady EW. Sentinel lymph node mapping following neoadjuvant chemotherapy for breast cancer. Breast J 2002; 8:97–100.
47. Breslin TM, Cohen L, Sahin A, et al. Sentinel lymph node biopsy is accurate after neoadjuvant chemotherapy for breast cancer. J Clin Oncol 2000; 18:3480–3486.
48. Stearns V, Ewing CA, Slack R, Penannen MF, Hayes DF, Tsangaris TN. Sentinel lymphadenectomy after neoadjuvant chemotherapy for breast cancer may reliably represent the axilla except for inflammatory breast cancer. Ann Surg Oncol 2002; 9:235–242.
49. Mamounas EP. Sentinel lymph node biopsy after neoadjuvant systemic therapy [review]. Surg Clin North Am 2003; 83:931–942.
50. Schwartz GF, Meltzer AJ. Accuracy of axillary sentinel lymph node biopsy following neoadjuvant (induction) chemotherapy for carcinoma of the breast. Breast J 2003; 9:374–379.
51. Sabel MS, Schott AF, Kleer CG, et al. Sentinel node biopsy prior to neoadjuvant chemotherapy. Am J Surg 2003; 186: 102–105.
52. Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol 1997; 15: 2483–2493.
53. Andea AA, Wallis T, Newman LA, Bouwman D, Dey J, Visscher D. Pathologic analysis of tumor size and lymph node status in multifocal/multicentric breast carcinoma. Cancer 2002; 94:1383–1390.
54. Anderson BO. Sentinel lymphadenectomy in breast cancer: an update on the NCCN Clinical Practice Guidelines. J Natl Compr Cancer Network 2003; 1:S64–S70.
55. Reintgen D, Giuliano R, Cox G. Lymphatic mapping and sentinel lymph node biopsy for breast cancer. Cancer J 2002; 8:S15–S21.
56. Ozmen V, Muslumanoglu M, Cabioglu N, et al. Increased false negative rates in sentinel lymph node biopsies in patients with multifocal breast cancer. Breast Cancer Res Treat 2002; 76:237–244.
57. Grant RN, Tabah EJ, Adair FE. The surgical significance of the subareolar plexus in cancer of the breast. Surgery 1959; 33:71–78.
58. Schrenk P, Wayand W. Sentinel-node biopsy in axillary lymph-node staging for patients with multicentric breast cancer. Lancet 2001; 357:122.
59. Kim HJ, Heerdt AS, Cody HS III, Van Zee KJ. Sentinel node drainage in multicentric breast cancers. Breast J 2002; 8:356–361.
60. Tousimis E, Van Zee KJ, Fey JV, et al. The accuracy of sentinel lymph node biopsy in multicentric and multifocal invasive breast cancers. J Am Coll Surg 2003; 197:529–535.
61. Kumar R, Jana S, Heiba SI, et al. Retrospective analysis of sentinel node localization in multifocal, multicentric, palpable, or nonpalpable breast cancer. J Nucl Med 2003; 44:7–10.
62. McMaster KM, Giuliano AE, Ross MI, et al. Sentinel-lymph-node biopsy for breast cancer—not yet the standard of care. N Engl J Med 1998; 339:990–995.
63. Ollila DW, Giuliano AE. Intraoperative lymphatic mapping and sentinel lymphadenectomy using isosulfan blue dye. Breast Dis 1998; 8:297–300.
64. Wong SL, Edwards MJ, Chao C, et al. The effect of prior breast biopsy method and concurrent definitive breast procedure on success and accuracy of sentinel lymph node biopsy. Ann Surg Oncol 2002; 9:272–277.
65. Miner TJ, Shriver CD, Jaques DP, Maniscalco-Theberge ME, Krag DN. Sentinel lymph node biopsy for breast cancer: the role of previous biopsy on patients eligibility. Am Surg 1999; 65:493–499.
66. Schnitt SJ, Silen W, Sadowsky NL, et al. Ductal carcinoma in situ. N Engl J Med 1988; 318:898–902.
67. Pendas S, Dauway E, Giuliano R, Ku NN, Cow CE. Sentinel node biopsy in DCIS patients. Ann Surg Oncol 2000; 7:15–20.
68. Klauber-DeMore N, Tan LK, Liberman L, et al. Sentinel lymph node biopsy: is it indicated in patients with high-risk ductal carcinoma-in-situ and ductal carcinoma-in-situ with microinvasion? Ann Surg Oncol 2000; 7:636–642.
69. Cox CE, Nguyen K, Gray RJ, et al. Importance of lymphatic mapping in ductal carcinoma in situ (DCIS): why map DCIS? Am J Surg 2001; 67:513–519.
70. Intra M, Veronesi P, Mazzarol G, et al. Axillary sentinel lymph node biopsy in patients with pure ductal carcinoma in situ of the breast. Arch Surg 2003; 138:309–313.
71. Carter BA, Jensesn RA, Simpson JF, Page DL. Benign transport of breast epithelium into axillary lymph nodes after biopsy. Am J Pathol 2000; 113:259–265.
72. Greenlee RT, Murray T, Bolden S, et al. Cancer statistics 2000. CA Cancer J Nlin 2000; 50:7–33.
73. Salvadori B, Saccozzi R, Manzari A, Andreola S, Conti RA, Cusumano F, Grassi M. Prognosis in breast cancer in males: an analysis of 170 cases. Eur J Cancer 1994; 7:930–935.
74. Maijer-van Gelder ME, Look MP, Bolt-de Vries J, Peters HA, Kijn JGM, Foekens JA. Clinical relevance of biologic factors in male breast cancer. Breast Cancer Res Treat 2001; 68: 249–260.
75. Rush Port E, Fey JV, Cody HS, Borgen PI. Sentinel lymph node biopsy in patients with male breast cancer. Cancer 2001; 91:319–323.
76. Gennari R, Renne G, Travaini L, Bassi F, Zurrida S. Sentinel node biopsy in male breast cancer: future standard treatment? Eur J Surg 2001; 167:461–462.
77. Woo JC, Yu T, Hurd TC. Breast cancer in pregnancy: a literature review. Arch Surg 2003; 138:91–99.
78. Nicklas A, Baker M. Imaging strategies in pregnant cancer patients. Semin Oncol 2000; 27:623–632.
79. Morita ET, Chang J, Leong SP. Principles and controversies in lymphoscintigraphy with emphasis on breast cancer. Surg Clin North Am 2000; 80:1721–1739.
80. Fenaroli P, Tondini C, Motta T, Virotta G, Personenin A. Axillary sentinel node biopsy under local anesthesia in early breast cancer. Ann Oncol 2000; 11:1617–1618.
81. Viale G, Bosari S, Mazzarol G, Galimberti V, Luini A, Veronesi P, Paganelli G, Bedoni M, Orvieto E. Intraoperative examination of axillary sentinel lymph nodes in breast carcinoma patients. Cancer 1999; 85:2433–2438.
82. Chilosi M, Lestani M, Pedron S, Montagna L, Benedetti A, Pizzolo G, Menestrina F. A rapid immunostaining method for frozen sections. Biotech Histochem 1994; 69:235–239.
83. Hermanek P, Hutter RVP, Sobin LH, Wittekind C. International Union Against Cancer. Classification of isolated tumour cells and micrometastasis. Cancer 1999; 86:2668–2673.

84. International (Ludwig) Breast Cancer Study Group. Prognostic importance of occult axillary lymph node micrometastases from breast cancer. Lancet 1990; 335:1565–1568.
85. Dowlatshahi K, Fan M, Snider HC, Habib FA. Lymph node micrometastases from breast carcinoma. Reviewing the dilemma. Cancer 1997; 80:1188–1197.
86. Cote RJ, Peterson HF, Chaiwun B, Gelber RD, Goldhirsch A, Castiglione-Gertsch M, Gusterson B, Neville AM. Role of immunohistochemical detection of lymph-node metastases in management of breast cancer. Lancet 1999; 354:896–900.
87. Chu KU, Turner RR, Hansen NM, Brennan MB, Bilchik A, Giuliano AE. Do all patients with sentinel node metastasis from breast carcinoma need complete axillary node dissection? Ann Surg 1999; 229:536–541.
88. Reynolds C, Mick R, Donohue JH, Grant CS, Farley DR, Callans LS, Orel SG, Keeney GL, Lawton TJ, Czerniecki BJ. Sentinel lymph node biopsy with metastasis: can AD be avoided in some patients with breast cancer? J Clin Oncol 1999; 17:1720–1726.
89. Viale G, Maiorano E, Mazzarol G, Zurrida S, Galimberti V, Luini A, Renne G, Pruneri G, Maisonneuve P, Veronesi U. Histologic detection and clinical implications of micrometastases in axillary sentinel lymph nodes for patients with breast carcinoma. Cancer 2001; 92:1378–1384.
90. Turner RR, Chu KU, Qi K, Botnick LE, Hansen NM, Glass EC, Giuliano AE. Pathologic features associated with nonsentinel lymph node metastases in patients with metastatic breast carcinoma in a sentinel lymph node. Cancer 2000; 89:574–581.
91. Masuda N, Tamaki Y, Sakita I, Ooka M, Ohnishi T, Kadota M, Aritake N, Okubo K, Monden M. Clinical significance of micrometastases in axillary lymph nodes assessed by reverse transcription-polymerase chain reaction in breast cancer patients. Clin Cancer Res 2000; 6:4176–4185.
92. Bostick PJ, Chatteriee S, Chi DD, Huynh KT, Giuliano AE, Cote R, Hoon DS. Limitations of specific reverse-transcriptase polymerase chain reaction markers in the detection of metastases in the lymph nodes and blood of breast cancer patients. J Clin Oncol 1998; 16:2632–2640.
93. Bostick PJ, Huynh KT, Sarantou T, Turner RR, Qi K, Giuliano AE, Hoon DS. Detection of metastases in sentinel lymph nodes of breast cancer patients by multiple-marker RT-PCR. Int J Cancer 1998; 79:645–651.
94. Kataoka A, Mori M, Sadanaga N, Ueo H, Tsuji K, Ray Y, Barnard GF, Sugimachi K. RT-PCR detection of breast cancer cells in sentinel lymph nodes. Int J Oncol 2000; 16:1147–1152.
95. Manzotti M, Dell'Orto P, Maisonneuve P, Zurrida S, Mazzarol G, Viale G. Reverse transcription-polymerase chain reaction assay for multiple mRNA markers in the detection of breast cancer metastases in sentinel lymph nodes. Int J Cancer 2001; 95:307–312.