The concept of sentinel lymph node (SLN) biopsy in breast cancer patients is simple, attractive and rapidly emerging as a new standard of care. Several aspects of the technique of lymphatic mapping, case selection, pathologic analysis and the finding of micrometastases, and the accuracy of the technique are important subjects of study and debate in the literature and will be discussed in this review. High identification rates can be attained by the use of both radioguided and blue dye lymphatic mapping. Intradermal injection of tracers has reported to be successful, suggesting that dermal and parenchymal lymphatics drain to the same SLN. Extra axillary drainage is only seen after peri- or intratumoural injection. SLN biopsy is most widely used for both palpable and non-palpable T1 and T2 tumours, and limited experience exists for other indications. Accuracy is high only in experienced hands. The impact of failure of the procedure on regional disease control and survival will be assessed in a trial of the NSABP (National Adjuvant Breast and Bowel Project). The influence of a positive SLN biopsy with and without axillary dissection on survival and local control will be studied in trials of the BASO (British Association of Surgical Oncology), ACOSOG (American College of Surgeons Oncology Group) and EORTC (European Organisation for Research and Treatment of Cancer). These phase III trials and related studies on the importance of micrometastases in the SLN will give new insights in the safety of the SLN procedure and in the importance of treatment of regional lymph nodes in relation to local disease control and survival.

Key words: breast cancer, case selection, lymphatic mapping, micrometastases, sentinel node biopsy

**Introduction**

The concept of sentinel lymph node (SLN) biopsy in breast cancer patients is simple, and therefore appealing. It concerns the identification and subsequent resection of the initial lymph nodes (‘sentinel nodes’) upon which the primary tumour drains. These nodes can be identified by radioguided lymphatic mapping and/or by visualisation of the nodes with vital blue dyes. The sentinel nodes are those most likely to contain tumour cells that have spread from the tumour. Histopathological evaluation of these nodes therefore can be an accurate predictor of other metastases in the same lymph node basin, and can guide regional and systemic treatment. Axillary node dissection and its morbidity can be avoided in patients in whom the sentinel nodes prove to be negative.

The concept is attractive and rapidly emerging as a new standard of care [1]. Knowledge of theoretical and clinical issues concerning the SLN procedure has been accumulating over recent years; however, a number of questions remain unanswered and are the subject of study and intense debate in the literature. In this review, some of the most relevant clinical issues will be discussed.

**Technical aspects of lymphatic mapping**

A number of techniques for identifying the sentinel node are applied. Overall, radioisotope mapping of the SLN with the use of a handheld gammaprobe succeeds more often than blue dye. Of 39 peer-reviewed pilot studies reporting the results of SLN biopsy validated by a ‘backup axillary lymph node dissection (ALND)’ using radioisotope (15 studies) or blue dye (20 studies) or a combination of both (11 studies), identification rates were 92%, 81% and 93%, respectively, while false-negative rates were 7%, 9% and 5%, respectively [1]. There is growing evidence that with the use of both methods in combination—blue dye and radioisotope—very high identification rates can be reached and fewer SLN may be missed compared with single-agent mapping [2]. In a multi-institutional study, injection of blue dyes alone was associated with decreased identification rates and a trend toward a higher false-negative rate [3]. The lower false-negative rate in dual agent tracing was hypothesised to be from the increased ability to identify multiple sentinel nodes. The same group demonstrated indeed that the false-negative rate was 14.3%
when single sentinel nodes were removed, and 4.3% for patients with more than one SLN removed. A logistic regression analysis of the 1287 SLN biopsy procedures showed that the use of blue-dye injection alone was the only factor independently associated with identification of a single SLN [4]. With the increase of experience, the additional value of blue dye localisation may decline. The proportion of positive sentinel nodes identified by blue dye only declined from 12% to 2% after 2000 SLN procedures using intradermal injected radioisotope and peritumoural patent blue [5].

Both for isotopes and for blue dyes, different routes of tracer administration in different combinations have been used [6]. The injection place can be related to either the skin (periareolar, subareolar, intradermal or subcutaneous over the primary tumour site) or the primary tumour (peritumoural or intratumoural). Groups at most centres inject around the tumour, but cutaneous methods are increasingly popular [3].

The skin-related methods are based on the hypothesis that the mammary gland and the overlying skin share the same lymphatic drainage. This was suggested in studies using peritumoural radioactive tracer injection and intradermal patent blue injection [7, 8], showing a high concordance in lymphatic drainage pattern between the two methods. Intradermal injection has the advantage that lymphatic vessel density in the skin is high, and tracers are cleared more rapidly and can be easily visualised by lymphoscintigraphy, which distinguishes better between first and second echelon nodes [9]. Linehan used unfiltered Tc-99m sulfur colloid injected into a single site in the skin over the tumour and peritumoural patent blue. There was a 95% concordance in drainage to the same node and the identification rate was 100% [2]. Multivariate analysis of 966 consecutive patients treated at the Memorial Sloan Kettering Center confirmed that intradermal injection is associated independently with a higher rate of successful sentinel node identification [10]. Also, the results of a large study in a multi-institutional setting of McMaster are in favour of the dermal isotope injection technique, with an identification rate of 89.9% and a false-negative rate of 8.3% for peritumoural isotope injection, and of 98% and 6.5% for the dermal technique, respectively, although the false-negative rates of the two methods were not significantly different [3].

Subdermal injection over the primary tumour [11, 12] has also shown good identification rates.

Despite good identification results, other authors state that these approaches do not provide the certainty that the lymphatics draining the skin or subcutaneous tissue are in all cases the same as lymphatics draining the tumour, based on anatomical studies of lymph drainage [6]. There are no randomised studies comparing the skin related to parenchymal injection techniques. Although axillary drainage is the principle drainage path of the breast, drainage from any quadrant of the breast can occur to other lymph node basins [13]. Extra-axillary drainage patterns are reported to be seen only after peri- or intratumoural radiocolloid injection [14]. The rate of visualisation of internal mammary nodes is in an average of 6–26% of mapped patients [14, 15]. Other less frequently (2–7%) identified drainage pathways are to supraclavicular, intrapectoral or intramammary nodes [6].

The overall and isolated internal mammary node involvement with metastasis is 23% and 5%, respectively, in patients undergoing SLN biopsy of internal mammary nodes. Cserni and Pap calculated that for all patients selected for lymphatic mapping, internal mammary node involvement is <5% overall, and ~1% for internal mammary node involvement without axillary disease [14]. Biopsy of internal mammary nodes is technically demanding, with a successful identification rate of 69% compared with 97% for axillary identification in one series [15]. An intensive review on the subject of the management of internal mammary node metastases was published by Klauber-DeMore et al. [16]. Metastases in internal mammary chain nodes are known to be a prognostic factor, but it is unknown whether upstaging by internal mammary SLN biopsy to select patients for adjuvant radiotherapy to the internal mammary chain will result in better local control or survival. The use of internal mammary chain SLN biopsy may have prognostic value when information obtained could change therapy, as in patients with negative axillary nodes and a primary tumour that does not select them for adjuvant systemic treatment (<1 cm). However, the proportion of these patients is expected to be very small and internal mammary chain mapping is recommended to be used only in the context of further studies on these topics in centres that have enough experience of SLN biopsy [14, 16].

From this point of view, one may chose to inject superficially (intradermally) if the aim of lymphatic mapping is to reveal only axillary sentinel nodes to avoid axillary dissection in node-negative patients. Peri- or intratumoural injection is more accurate at performing staging of other nodal basins such as internal mammary chain nodes or staging of deep tumours, which may not share the same lymphatic pathway as the skin [6].

There is a wide variation in the isotope techniques used concerning choice and dosage of isotope, carrier, particle size, timing of injection, and definition of a successful result. The general similarity of outcome despite high variations in technique indicates that SLN biopsy is a generally robust procedure. However, the optimum technique remains to be established.

Lymphoscintigraphy can be very helpful to localise SLN pre-operatively, especially when lymph node basins other than the axilla need to be explored. However, for axillary identification of SLN, its additional value above handheld gammaprobe identification has been discussed, as two studies demonstrated no additional benefit of lymphoscintigraphy for SLN identification in the axilla [17, 18].
Case selection

Most of the reported experience with SLN biopsy includes patients with clinical stage T1–2N0 [1]. The procedure seems equally accurate for T1 and T2 tumours [19, 20]. Experiences with sentinel node procedures of T3 tumours are limited, but seem to be associated with increased false-negative rates [21].

It has been hypothesised that if lymphatics and lymph nodes become progressively infiltrated with tumour cells, alternative pathways of lymph drainage may occur, or the nodes may no longer be able to retain tumour cells and the sentinel nodes will not be visualised during lymphoscintigraphy [9]. Clinically suspicious non-SLNs were present in >50% of false-negative SLN biopsy procedures in the Memorial Sloan Kettering Cancer Centre, and careful intraoperative palpation of the axilla is therefore an essential component of SLN biopsy procedure, especially when a high chance of nodal involvement is suspected [1]. Any suspicious nodes should be removed for histopathological evaluation [22].

SLN biopsy has been used in patients with ductal carcinoma in situ (DCIS). In a group of 87 patients with pure DCIS lesions, Pendas found 5 patients (6%) with SLN metastasis. Routine haematoxylin–eosin (H&E) staining detected the metastasis in two of these five patients and immunohistochemistry (IHC) detected it in all five patients. Four patients had high-grade DCIS and one patient had a 9.5 cm lesion of low-grade DCIS. ALND was performed in all five patients and the SLN were the only nodes found to harbour metastases [23]. In another study of 76 patients with high-risk DCIS, nine patients (12%) had positive SLNs and seven out of nine were positive for micrometastases only. Six patients out of nine had a completion axillary dissection, and one of the patients with a SLN micrometastasis had an additional positive node on routine H&E sectioning. Of 31 patients with DCIS with microinvasion, three (10%) had positive SLN and two out of three were positive for micrometastases only [24]. However, there is no consensus about the indication of SLN biopsy for DCIS. The significance of micrometastases in the sentinel node from a true DCIS is questioned by DCIS experts [25]. In a series of 103 cases of DCIS, treated between 1974 and 1992 with negative nodes on routine sections, additional serial step sections and immunohistochemistry increased the number of lymph node metastases to 12 (12%), all of which were micrometastases. Clinical follow-up revealed 12% recurrence, none of which involved the micrometastasis-positive group [26].

The results regarding the use of SLN biopsy after neoadjuvant chemotherapy are inconsistent and based on small numbers. Fernandez et al. [27] and Nason et al. [21] found false-negative rates of 22% and 33% in 36 and 33 mapped patients, respectively, while in other studies better results were reported, with no false-negatives in 33 mapped patients [28], zero in 29 mapped patients [29] and three in 43 mapped patients [30]. SLN biopsy can be used without surgical removal of the tumour before neo-adjuvant chemotherapy in order to guide the choice for locoregional treatment after completion of the systemic treatment. In the setting of advanced disease, SLN biopsy may play a role in estimating the response to neoadjuvant chemotherapy [31].

There is no restriction for using SLN biopsy in non-palpable tumours. Techniques may vary but the most minimally invasive approach is percutaneous imaging-guided (stereotactic or sonographic guidance) diagnosis of the primary tumour, and removal of the tumour and sentinel lymph nodes in one session. A single surgical procedure is then possible in 82% (164 out of 200) of carcinomas [32]. Peritumoral injection of radioisotope or blue dye can be guided using the wire needle localisation. If breast cancer is diagnosed with excisional biopsy, no influence of excision volume or tumour location on the identification rate or false-negative rate of SLN was reported in one series of 181 excised tumours [33]. Large upper quadrant biopsy cavities may preclude accurate staging by disruption of the axillary lymphatics [1]. There are a number of patient categories (male patients, elderly patients, patients with multifocal tumours and those requiring prophylactic operations) where SLN biopsy has only been used to a limited extent and literature on this subject has not yet been widely published.

Multifocal tumours are an exclusion criterion for performing SLN biopsy in most centres. Satisfying results in small numbers [19] of such patients have been described [34].

SLN biopsy has been shown to be successful in a small group of 16 male patients treated at the Memorial Sloan Kettering Cancer Center. Mean tumour size was 1.3 cm, and SLNs were identified in 15 out of 16 patients (93.75%). There were 10 patients with negative nodes, of whom six had a mean of 5.7 additional nodes removed, all of which were negative [35].

Increasing patient age affects successful lymphatic mapping. Not only are fewer sentinel nodes found, they are also found less frequently [36]. Krag suggested that this was secondary to the progressive replacement of the parenchyma of the lymph nodes by fat [37]. A higher false-negative rate has not been found [38], but the reported numbers are small.

In patients selected for prophylactic mastectomy, SLN biopsy has been used as a diagnostic procedure as the mastectomy precludes the subsequent option of SLN biopsy in case an unsuspected carcinoma is found. Of 57 patients treated using these procedures, four (7%) experienced a significant change in their surgical management: in two patients carcinoma was found in the breast but they had negative sentinel nodes, and another two had positive sentinel nodes by immunohistochemistry with no tumour in the breast. These latter patients subsequently underwent a complete axillary dissection and no additional metastatic nodes were found [39].
**Pathological analysis and micrometastases**

In the past, many authors have reported finding micrometastases that were not found by routine section of the lymph nodes, but were identified only by serial sectioning and immunohistochemical staining. The increased yield of serial sectioning with haematoxylin–eosin (H&E) staining has been reported as ranging from 7% to 33% [40]. The Ludwig Breast Cancer Study Group showed that micrometastatic nodal disease, which was present in 9% of cases (detected by serial sectioning and H&E staining), affected both disease-free survival and overall survival after 6 years of follow-up [41]. A recent update of the same patient population confirmed that occult lymph node metastases detected using H&E and also IHC were associated with worse disease-free and overall survival in post-menopausal women [42].

The major disadvantages of introducing this approach in clinical practice were cost and labour implications in processing all axillary lymph nodes through serial sectioning. The SLN biopsy offers the possibility of examining lymph nodes in great detail without these drawbacks [43].

Histopathological evaluation by taking step sections at regular intervals increases the percentage of metastases found in SLNs by ~10% [44]. The exact number of step sections and step size needed to reveal all metastatic foci in one node is unknown and methods differ between centres, but a section interval of 0.25 mm is proposed as a practical and accurate approach [45, 46].

The use of immunohistochemistry for detection of tumour cells in nodes negative according to H&E staining remains controversial. Detection of occult micrometastases is of prognostic significance for the presence of metastases in non-sentinel nodes. In 93 patients whose sentinel node metastases were <2.0 mm, 24 (26%) had non-SN metastases, which included seven macroscopic, six H&E microscopic and 11 IHC metastases. The majority (63%) of the 101 patients with SLN macrometastases had non-SLN metastases. Multivariate analysis demonstrated that the size of the SLN metastasis, extranodal hilar tissue invasion, tumour size and peritumoural lymphatic vascular invasion were correlated with the presence of non-SLN metastases [47]. In another study, also using IHC for both SLNs and non-SLNs, patients with a single positive SLN and patients with metastases <1 mm² in the SLN had significantly less non-SLN involvement than patients with more than one positive node (40% compared with 78%, respectively) and patients with macrometastases (27% and 49%, respectively) [48]. In the whole group of SLN-positive patients, ~50% had metastases in non-SLNs at subsequent ALND [49]. However, in the group of patients with a positive SLN, no subset of patients could be identified conclusively without SLN metastases.

The benefit of the completion axillary lymphadenectomy in patients with (micro)metastases in SLNs is unknown and questionable because of the controversial role of locoregional control for survival and the effect of adjuvant systemic therapy and radiotherapy on residual disease in the axilla. Trial Z0011 of the American College of Surgeons Oncology Group (ACOSOG) addresses the clinical importance of the metastases in the non-SLN, and the impact of ALND for SLN-positive patients on survival. In this trial, women with T1 and T2 N0M0 breast cancer and a positive SLN were randomised between ALND or observation of the axilla [50]. Primary end point is overall survival, and secondary end points are surgical morbidities and distant disease-free survival.

An ongoing European EORTC trial called AMAROS (After Mapping of the Axilla Radiotherapy Or Surgery) has been designed to determine the effectiveness of axillary radiation in comparison to ALND and the associated morbidity of both treatment modalities [51].

Once these trials have been concluded, it will be established whether SLN biopsy alone or SLN biopsy followed by radiotherapy provide the same therapeutic benefits as ALND but without the surgery-associated morbidity.

The influence of the upstaging of patients by finding micrometastases with thorough examination of SLNs on overall survival by the wider use of adjuvant chemotherapy is unknown. Immunohistochemistry detects metastases in 12–29% of patients with T1a/1b tumours who are node negative on H&E staining, and may be used to recommend adjuvant systemic therapy only because of these micrometastases [52]. The ACOSOG Z0010 trial is a prospective evaluation of the significance of bone marrow and SLN micrometastases in SLN-negative patients processed using H&E [50]. In previous studies, the presence of bone-marrow micrometastases was associated with shorter relapse-free and overall survival, but was not an independent prognostic factor in multivariate analysis after a median follow-up of 12.5 years [53].

Reverse transcriptase–polymerase chain reaction of mRNA only expressed in cancer cells has even more potential to detect single groups of cancer cells, but there are still methodological problems, and the clinical significance of single cells that escape extensive histopathological investigation remains to be established [45].

**Accuracy of SLN biopsy**

The success of SLN biopsy is hampered by false-negative procedures: the wrong node may be removed and nodes containing metastases may remain unrecognised in patients. This can be detrimental because it may adversely affect local control, and result in understaging of the patient and underestimation of the need for adjuvant systemic treatment.

False-negative biopsies have a complex aetiology with patient- and tumour-related factors, such as re-routing of lymphatic flow by tumour blockage of the lymph node and variability in lymph flow, and also with non-patient-related factors such as pathologist sampling error and surgeon inexperience [22]. The last factor has been well recognised in the literature. It has been documented that there is a definite
learning curve for the procedure. Results representing the mean of five surgeons’ experiences in a one-centre setting indicate that 23 and 53 cases are required to achieve success rates of 90% and 95%, respectively, in identifying the sentinel node [54]. McMasters published the results of the largest prospective multi-institutional study to date, on 2148 patients with SLN biopsies performed by 226 surgeons. They showed that SLN identification rate and false-negative rates were significantly improved after the completion of 20 cases per surgeon [38]. However, skills are not only related directly to the number of procedures performed, but also appear to be related to the technical skills of the surgeon. Initial experiences published by Krag and colleagues of 11 surgeons participating in a multi-centre study showed that the success rates differed significantly among the participating surgeons. One surgeon had a minimum of a 79% identification rate, but a false-negative rate of 7% after 43 procedures, while another surgeon had a maximum of a 98% identification rate but a false-negative rate of 27% after 51 procedures [37]. Guidelines for successful implementation of the SLN biopsy have been published and include: the use of a formalised protocol, patients being fully informed, backup axillary dissection to validate the early experience, and audits of individual and institutional results [55, 56]. However, in a survey of 1000 randomly selected surgeons in the United States, a marked variation in the number of SLN biopsies validated before performing SLN biopsy was found: 28% of respondents performed ≤10 procedures with subsequent ALND before performing SLN biopsy alone [57]. The same was found in The Netherlands, where 43% of surgeons who had done <10 SLN biopsies felt themselves competent enough to omit ALND [58].

Although the sentinel node procedure has been proven to be valid in numerous studies, all studies report a definite number of false-negative procedures. The percentage of patients with false-negative results cannot be calculated using the total number of patients, as the majority of patients with T1 and T2 tumours are node-negative and SLN biopsy cannot be false-negative in these cases. The false-negative rate is defined as the number of false-negative procedures divided by the sum of the true-positive and false-negative procedures. Sensitivity is defined as the complement of it: the number of true positives divided by the sum of the true positives and false negatives. Obviously, sensitivity (and its associated confidence interval) depends only on the fraction of node-positive patients. In a meta-analysis of 18 phase I/II studies on 2500 sentinel node biopsies with backup axillary dissection assessing the validity of the technique, only studies with >50 node-positive patients were used [59]. Sensitivity ranged from 83% to 100% and pooled sensitivity was 91% (95% confidence interval = 89% to 93%). These authors concluded that SLN biopsy is a safe substitute of ALND. However, the possibility that ALND is not a perfect standard for comparison is also considered, as it may carry some risk for false-negativity for nodal disease on its own [59]. The extent of the overestimation of sensitivity by the use of conventional ALND (with routine H&E examination of one or two sections of each node) as a control for the SLN procedure in one-arm studies has been calculated by Roy and colleagues [60]. The true sensitivity declined from 94% to 85%, and the true negative predictive value declined from 97% to 91%, with 10% of true node-positive cases missed by SLN biopsy and ALND.

The influence of inaccuracy of SLN biopsy on axillary recurrence and survival is a complex matter and is currently unknown. It will be influenced by the benefits of the procedure, such as more adequate pathological nodal staging, against the pitfalls of understaging patients, omitting adjuvant therapy and failure to treat regional disease. Factors determining these figures are patient selection, the numbers of expected positive nodes and the wider use of adjuvant chemotherapy in node-negative patients.

There have been three follow-up studies of SLN-negative patients without subsequent treatment of the axilla. All have a short follow-up but demonstrate a low level of regional recurrence of 0% in 67 patients after a median of 39 months [61], 0% in 285 patients after a median of <24 months [62], and 1% in 100 patients after a median follow-up of 24 months [63].

The influence of SLN biopsy on regional disease control and quality of life will be addressed in the ALMANAC (Axillary Lymphatic Mapping Against Nodal Axillary Clearance) trial of the British Association of Surgical Oncology (BASO). Patients with clinically negative axillary nodes are randomised between SLN biopsy, followed by axillary surgical treatment only in cases of a positive sentinel node and conventional axillary surgical treatment [64]. In the National Adjuvant Breast and Bowel Project (B-32 trial) of the National Cancer Institute, patients in the United States with clinically negative axillary nodes will be randomised between SLN biopsy followed by ALND, and SLN biopsy followed only by ALND in cases of a positive SLN on H&E staining. All negative sentinel nodes will be processed by immunohistochemistry in a blinded evaluation [65].

These trials should provide the final proof that sentinel node biopsy is equivalent to ALND in regional disease control and patient survival.

At the consensus conference on the role of SLN biopsy in carcinoma of the breast in April 2001 (Philadelphia, USA), surgeons were strongly encouraged to perform SLN biopsy within the context of one of the ongoing American and European clinical trials [66].

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