A mini-review on factors and countermeasures associated with false-negative sentinel lymph node biopsies in breast cancer

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Abstract

Sentinel lymph node biopsy (SLNB) is a new surgical technique for local axillary lymph nodes (ALNs) of breast cancer. Large-scale clinical trials have confirmed that undergoing SLNB and ALN dissection (ALND) showed no significant difference for sentinel lymph node (SLN)-negative patients in terms of disease-free survival, overall survival and recurrence-free survival. However, false-negative results are still the main concern of physicians as well as patients who undergo SLNB instead of ALND. The American Society of Breast Surgeons established a task force to suggest acceptable standards for SLNB. In 2000, the task force recommended that the identification rate for SLNB be 85% or higher and the false-negative rate be 5% or lower. This review focuses on clinical factors (tumor volume, multifocal/multi-center cancers, neoadjuvant chemotherapy and skip metastasis), tracer techniques and pathological factors affecting SLNB and explores methods for reducing the false-negative rate.

Keywords: Breast cancer; sentinel lymph node biopsy; false-negative rate

Introduction

The sentinel lymph node (SLN) is the first lymph node to receive lymphatic drainage from a tumor, and also theoretically the first site of lymphatic metastasis. In the 1990s, SLN biopsy (SLNB) was developed as a surgical technique for local axillary lymph nodes (ALN) of breast cancer. This technique was a landmark in the development of breast surgery and has become an important part of the standard treatment for early breast cancer. Large-scale clinical trials have confirmed that SLNB and ALN dissection (ALND) show no significant difference in SLN-negative patients in terms of disease-free survival, overall survival and recurrence-free survival (1-3), and SLNB can accurately predict the metastasis of ALNs. In principle, SLN-negative patients can be free from ALND, however, the issue of false-negatives remains the main obstacle for doctors and patients who receive SLNB instead of ALND, and to a certain extent hinders the clinical promotion of SLNB. In 2014, the American Society of Clinical Oncology reported 6 trials of SLNB, in which the false-negative rate (FNR) was between 4.6% and 16.7% (4). Kim *et al*. (5) performed a meta-analysis and concluded that the average FNR of SLNB was 8.4% (0–29%). The issue of FNR has hindered the wide-scale application of SLNB in clinical practice. Therefore, the investigation of factors associated with FNR and strategies effectively reducing the FNR, has become a research focus in breast surgery.

Clinical factors and countermeasures

Effects of clinical factors on FNR of SLNB

Tumor volume

It is commonly believed that the FNR of SLNB is relatively low in small tumors. Pecha *et al*. (6) reported an FNR of 5% in patients with an original tumor smaller than 2 cm in size, 9%

for tumors between 2 and 4 cm in size, and 13.8% for tumors greater than 4 cm in size. Gimbergues *et al.* (7) detected an FNR of 5.7% in T1-T2 patients, but 28.5% in T3 patients (P=0.045), confirming the close correlation between the FNR of SLNB and tumor volume. It is therefore widely known that SLNB is mostly suitable for T1-T2 patients. In large tumors with an increased rate of lymphatic metastasis, the metastatic cancer cells often clog the lymphatic channels, which change the original lymphatic circulation, and thus hinder the normal transfer of the imaging agent or radionuclide in the lymphatic vessels. As a result, the bypassed agent is transferred to the lymph nodes without metastasis, leading to a higher FNR. Borgstein *et al.* (8) suggested that the higher FNR of SLNB in patients with massive tumors is associated with impaired lymphatic integrity. During early metastasis, the ability of lymph nodes to absorb the tracer is strong due to the high levels of activity of macrophages. When most or all of the lymph nodes are affected by the tumor, their ability to absorb the tracer is markedly reduced, and the tracer is drained along the lymphatic vessels to other lymph nodes, leading to an increased FNR. During surgery, swollen hard non-staining lymph nodes with pathologically confirmed metastases are frequently detected near the blue-stained SLNs, whereas blue-stained lymph nodes often have no metastases, which further supports the findings in the literature.

Multifocal cancer

Veronesi *et al.* (9) suggested that SLNB is not suitable for patients with multifocal/multi-center breast cancer. They proposed that each lesion may have an individual lymphatic drainage pathway, and that these pathways may sometimes be connected. An SLN-negative result for one lesion cannot assure negative results for other lesions. Several studies have recognized the breast as a whole organ, and SLNs are the lymph nodes of not only the tumor but also the whole organ. The anatomic position of SLNs is constant in multifocal breast tumors and some other types of tumors (10,11). Although the metastasis rate of SLNs and the rate of non-SLN biopsies in multifocal breast cancer are higher compared with unifocal breast cancer patients, the overall FNRs of SLNB in these two groups of patients are comparable (9,10). In a retrospective analysis of 932 multifocal/multi-center breast cancer patients who had undergone SLNB, the rate of accuracy and the FNR were 96.0% and 7.7%, respectively (12). A meta-analysis on 996 cases of multifocal/multi-center breast cancer revealed a success rate of 92.0%–100.0% and an FNR of 0–25.0% (13), which is close to that of unifocal breast cancer. Currently, the Chinese Anti-Cancer Association Committee of the Breast

Cancer Society (CACA-CBCS) still considers multi-center breast cancer as one of the indications of SLNB.

Neoadjuvant chemotherapy

Neoadjuvant chemotherapy (NAC) is mainly used for locally advanced breast cancer patients, in which it can reduce the tumor stage and the number of positive ALNs before surgical treatment. It has been shown that about one third of ALN-positive patients may become negative after NAC. This therapy was also shown to reduce the lymph node stage in approximately 55% of patients (14,15). However, there are some controversies over whether the FNR of SLNB is changed after NAC. Most scholars believe that the FNR of SLNB after NAC is much higher compared with the pre-treatment FNR, and thus it cannot be used to accurately evaluate the conditions of ALNs (6,16). Pecha *et al.* (6) found that the post-NAC SLNB cannot accurately detect the conditions of ALNs, yielding an FNR of up to 19.5%. The higher FNR has been a major issue of SLNB in advanced patients who have undergone NAC compared with early breast cancer patients without NAC. These scholars claim that NAC may change the drainage of ALNs and even destroy the lymphatic vessels connecting to SLNs. Consequently, the identified lymph node is not actually a real SLN. Moreover, NAC may cause the clogging of lymphatic vessels due to necrosis, fibrosis, inflammation and tumor emboli during the therapy, which prevents the dye and radioactive markers from reaching the SLNs. However, in recent years, an increasing number of studies have shown that the FNR is not affected by NAC, although the success rate of SLNB may be slightly reduced after therapy. The mean SLNB success rate and the FNR after NAC were 89% and 10%, respectively, which were close to previously reported rates for SLNB (17-19). Moreover, the conditions of SLN can reflect those of ALN. In addition, numerous reports have suggested that pre-NAC ALN, staging as an important factor, affect the FNR of SLNB after therapy (20,21). A study by Takahashi *et al.* (20) showed that the FNR was significantly lower in clinically node-negative patients than in node-positive patients before NAC (5.5% *vs.* 35.5%, P=0.001). Gimbergues *et al.* (7) conducted a study on 129 breast cancer patients who had undergone NAC before surgery, and found that the post-NAC FNR of SLNB in N_{1-2} patients (29.3%) was significantly higher than that of N_0 patients (0%, P=0.003). In another study of 3,746 $T_{1-3}N_0$ breast cancer patients, including 575 (15.3%) who had undergone SLNB after chemotherapy and 3,171 (84.7%) who had undergone surgery first, the SLN identification rates were 97.4% in the neoadjuvant group and 98.7% in the surgery first group (P=0.017). The FNRs of the neoadjuvant group and the

surgery first group were 5.9% and 4.1%, respectively (P=0.39). The study also suggested that the post-NAC SLNB does not increase the local recurrence rate, and SLNB is a reliable alternative to ALND in cN_0 patients after NAC (21). However, an NSABP B-27 study with a large sample size did not detect a significant difference in the FNR among cases with different ALNs conditions (P=0.51) (22). When patients undergo SLNB after NAC, they do not have to undergo surgery before and after NAC, which avoids the delay in chemotherapy. Furthermore, such a treatment strategy can allow the clinician to learn how the ALNs respond to chemotherapy. Clinical practice data have demonstrated the safety of SLNB after NAC in cN_0 patients (18,19). Although ALN-positive patients who become negative after NAC are considered to be indicative of SLNB, there are some controversies over this in the literature and further validation is needed in future studies.

Skip metastases

Skip metastases refer to the phenomenon in which high-level (Level II, III) lymph nodes are involved in cancer metastases without the involvement of low-level (Level I) lymph nodes. The incidence of skip metastases is between about 1.5% and 19.2% (23,24). SLNB can only detect Level I lymph nodes, and thus will yield FNRs in cases of skip metastases. Gaglia *et al.* (24) reported that Level I lymph node metastasis did not occur in 14.9% of ALN-positive patients, and approximately 1/7 of these patients could not be detected by SLNB or Level I lymph node clearance. The internal mammary lymph node is also the first lymph node affected by breast cancer metastasis. Metastatic cancer cells may skip Level I lymph nodes and directly affect Level II and III lymph nodes through the internal mammary lymph node chain. Conventional SLNB will yield false-negative results in these cases. Therefore, false-negative results from SLNB cannot be completely eliminated due to the occurrence of skip metastases and the limitations of current diagnostic technologies. The American Society of Breast Surgeons has recommended that SLNB, with a detection rate of above 85% and an FNR below 5%, can only be considered as a safe alternative to ALND (25). The development of strategies to reduce the FNR to an acceptable level is an area of current research focus.

Measures to reduce FNR

Strictly following indications

In 2015, the CACA-CBCS noted that the absolute contraindications of SLNB were inflammatory breast cancer and N_2 breast cancer only. Patients with clinically suspicious ALN enlargement who were cytologically or pathologically negative after fine-needle aspiration or core needle biopsy could undergo the SLNB procedure. Although breast and axillary surgery, radiotherapy and NAC may change the original condition of the lymphatic circulation and affect the lymphatic metastasis pathway leading to FNRs in SLNB, their effect on the FNR is small. Therefore, surgery, radiotherapy and NAC are relative contraindications of SLNB. However, SLNB is not recommended to patients with non-tumor factors such as a prior history of breast reduction surgery, breast augmentation and breast reconstruction due to the risk of high FNR. With advances in research on SLNB, an increasing number of relative contraindications have become indications. Clinicians should make flexible, appropriate choices regarding SLNB after thoroughly reviewing each patient's case.

Learning curve

SLNB is a technology requiring strong operational skills. The FNR of SLNB is associated with the experience level of clinicians. The FNR is lower when SLNB is performed by clinicians with more experience of this technique, and the FNR should become consistent when a clinicians' experience reaches a certain level. This process is usually called "the learning curve". Cox *et al.* (26) believed that a clinician can only master the technology after the completion of 20 independent cases of SLNB, whereas Snider *et al.* (27) claimed that experience of 45 cases of SLNB is required. If the identified SLNs are not actually SLNs or if only some of the SLNs are detected, the FNR will be increased. Therefore, false-negative cases of SLNB often occur in the early learning stages. The CACA-CBCS has recommended that ALND should not be replaced by SLNB until a clinician has independently performed more than 40 cases of SLNB followed by ALND and reached an SLNB success rate of 90% and an FNR of less than 10%.

Increase number of detected lymph nodes

In addition to strictly following the indications and completing the learning curve, appropriately increasing the number of detected SLNs is undoubtedly the most effective measure to reduce the FNR of SLNB. The NSABP B-32 trial showed that the FNR is significantly reduced as the number of detected SLNs increases. The FNRs associated with the detection of 1, 2 and 3 SLNs were 18%, 10% and 7%, respectively (18,28). In another clinical trial, ACOSOG Z1071, the FNRs of 2 and ≥3 SLNs were 21.1% and 9.1%, respectively (P=0.007). In addition, in patients where a radiopaque clip had been placed in the positive node at the time of biopsy, when this node was identified and removed as part of the sentinel node procedure, the FNR was <7% (18,19). The SLNs of 596 patients with

breast cancer were examined using radiocolloids with a blue dye tracer in Shandong Cancer Hospital affiliated to Shandong University between March 1, 2012 and June 30, 2015. First, the SLNs were removed, and then, the area surrounding the original SLNs was selected, and the lymph nodes visible in a field 3–5 cm in diameter around the center were removed. Finally, ALND was performed. For patients with ≤3 detected SLNs, peripheral lymph node sampling has been reported to reduce the FNR of SLNB to an acceptable level of less than 5% (29). Therefore, for patients with few detected SLNs, the anatomical area around the SLNs should be expanded to allow detection of more lymph nodes, which may be an effective approach to enhance the detection rate and reduce the incidence of local treatment failures.

Factors relating to tracer techniques and countermeasures

Effect of tracer techniques on FNR of SLNB

The CACA-CBCS has noted a similar FNR for SLNB when three of the most common blue dye tracers are used: isosulfan blue, patent blue and methylene blue. Radionuclide tracers include ^{99m}Tc-labeled sulfur colloid, antimony colloid and protein colloid. Studies have shown that the use of small-molecule radioactive colloids can increase the number of detected SLNs, but do not affect the success rate and FNR of SLNB (30). Wong *et al.* reported FNRs of 11.9%, 11.8% and 7.3% for the dye, radionuclide and combined method, respectively. There was no significant difference in the FNR between the combined method and each individual tracer (P=0.058) (31). It was conventionally believed that the SLNs related to a primary tumor could only be detected by injection of tracers around the tumor. However, anatomical studies have found that the density of the lymphatic vessels in the breast skin is higher than that of the parenchyma, and therefore the success rate and FNR of SLNB with intradermally or subcutaneously injected tracers are theoretically superior to the glandular injection approach. Recent studies have shown that the SLNs are the lymph nodes of not only the tumor but also the whole breast. The detection rate and FNR of SLNB for different injection sites were found to be almost identical (10,11). In a prospective, multi-center study on 3,961 breast cancer patients by Chagpar *et al.*, no significant difference was detected in the success rate and FNR among SLNB with tracers injected at a variety of sites including the gland, intradermal and subcutaneous sites, and the areola area (32). Regarding the injection time of the tracer, it is recommended that the blue dye be injected 10–15 min before the surgery and the radionuclide 3–18 h before surgery. The CACA-CBCS indicated that the

use of 220 nm filtered sulfur colloid does not affect the FNR of SLNB. In clinical practice, the time from tracer injection to detection is usually 2–4 h in cases where filtered sulfur colloid is used, whereas the time interval between the injection of unfiltered sulfur colloid and SLNB is longer. The unfiltered sulfur colloid is sometimes injected a day prior to biopsy.

An ideal tracer should be stable. In other words, the tracer should rapidly accumulate at the first lymph node station instead of the second and third stations. Recently, carbon nanoparticles and fluorescent dyes, the third generation tracers, have emerged in the clinical tracing field. Nanocarbon particles are not easily taken up by capillary vessels due to their size (about 150 nm), and instead they have strong lymphatic tropism and can enter the capillary lymphatic vessels. These particles are then drained into the SLNs where they become stained black due to phagocytosis by macrophages. Studies have suggested that carbon nanoparticles are superior to blue dye in terms of reducing the FNR in SLNB (33). Localization techniques using non-radioactive tracers warrant investigation and there is increasing evidence to support the efficacy of indocyanine green (ICG) fluorescence as part of a dualtechnique, either combined with blue dye or radioisotope, for SLN identification. The illuminated subcutaneous lymphatic channels can be seen on a photodynamic eye (PDE) camera display and ICG can be tracked as it passes towards the axilla. The PDE method is not perfect because mechanical obstruction due to tumor embolism in the lymphatic channel or inflammation cannot allow ICG fluorescence in SLNs. In contrast, the ICG fluorescence-guided method only increases the detection rate of SLNB, but does not significantly reduce the FNR when compared with conventional tracers (34,35). Lymphoscintigraphy is a common method for the preoperative imaging of SLNs in the axillary and internal mammary area, which can illustrate the number and location of SLNs and is especially useful in the detection of non-axillary SLNs. The technique has provided a reliable reference for intraoperative detection of SLNs.

Countermeasures

Once the learning curve is completed, the SLNB is accurate regardless of the tracer used. It has been widely accepted that the FNR in SLNBs using combined tracers is much lower than that in SLNBs using a single tracer (36). A multi-center study showed that the FNR in SLNBs using combined tracers was reduced by 2.5% compared with the single tracer group, and the success rate of the former approach was increased by 1.3% (37). Wong *et al.* reported that the single tracer approach was more efficient in the detection of one SLN (P<0.0001), whereas

the combined tracer was more useful in the identification of multiple SLNs (31). Since increasing the number of detected SLNs is undoubtedly the most effective method to reduce the FNR of SLNBs, it highly recommended that the combined tracer of dye and radionuclide should be used in the procedure. A single tracer may be used under conditions whereby the combined tracer is not available. In the radionuclide-guided SLNB, the detector is placed close to the target and moved slowly during the procedure. In the SLNB using blue dyes, it is recommended that the site should be massaged for 3–5 min after the injection, and each blue-stained lymphatic vessel should be dissected to increase the detection rate. The results of Wang *et al*. (38) revealed three types of sentinel lymphatic channels (SLCs), including superficial SLC (SSLC), deep SLC (DSLC) and penetrating SLC (PSLC), and six lymphatic drainage patterns based on the three types of SLCs, including SSLC, DSLC, PSLC, SSLC+DSLC, SSLC+PSLC, and DSLC+PSLC. The proportions of the drainage patterns were 43.0%, 0.9%, 15.9%, 33.6%, 3.7% and 2.8%, respectively. If only one or two SLNs instead of all blue-stained lymphatic vessels were pathologically examined, some or all metastatic SLNs may be missed, resulting in an increased FNR. Therefore, a successful SLNB requires extensive experience, commitment and patience by clinicians. The axillary region should be palpated after SLNs are detected, and enlarged hard lymph nodes can be considered as the lymph nodes surrounding the SLNs and should be individually examined. Moreover, different strategies should be adopted in patients showing different characteristics. For instance, when the tumor is located in the upper outer quadrant of the breast, especially close to the axilla, the tracer should be injected beneath the areola instead of by the peritumoral method to prevent interference of the γ-ray emitted by the injection point on the detection of SLNs. For older patients, the failure rate of gland tracer injection is relatively high compared with other patients due to atrophy of the gland and increased levels of fat, leading to a higher FNR. In these cases, superficial injection or the combination of superficial and glandular injection may be superior to glandular injection.

Pathological factors and countermeasures

The SLN metastases include macro-metastases (T>2.0 mm), micro-metastases (0.2 mm<T≤2.0 mm) and isolated tumor cells. Typically, the target lymph nodes are divided into two parts, and the middle one or two layers are stained with hematoxylin and eosin. It is therefore difficult to find potential micro-metastases and isolated tumor cells, leading to high rates of false-negative results and misdiagnoses. The accurate identification of micro-metastases is critical during SLNB. Serial section (SS) technology can more effectively identify micro-metastases or nest-distributed isolated tumor cells in lymph nodes by providing substantially more layers. Studies have reported that micro-metastases are detected by SS in 5%–10% of lymph nodes that are negative by conventional pathological examination (39). Osako *et al*. (40) reported that almost all macro-metastases can be identified by SS at 1 mm intervals, and micro-metastases can be detected by SS at 200 μm intervals. Currently, reverse transcription polymerase chain reaction is the major, sensitive molecular method for the detection of micro-metastases, which can identify one metastatic cancer cell among 1×10^6 normal cells. However, this method is not suitable for wide clinical application due to possible non-specific false-positive results and high cost.

Conclusions

Research into false-negative SLNBs of breast cancer has facilitated more evidence-based medicine and enabled the development of new methods to reduce the FNR of SLNB. However, recently the medical model has changed from "evidence-based medicine" into "precision medicine". Future studies should focus on ways to maximally reduce the FNR of SLNB based on the specific characteristics of patients. With the increasing number of indications of SLNB, the demands on the technology are increasing to assure a high success rate and a low FNR, which will benefit patients. Refinements of SLNBs require not only the efforts of surgeons but also the cooperation of clinicians in radiology, nuclear medicine and pathology, which makes the SLNB the most reliable measure in axilla-conserving treatment.

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Footnote

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