Review Article

New techniques for sentinel node biopsy in breast cancer

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Abstract: Sentinel lymph node biopsy (SLNB) is still the standard of care for axillary nodal staging in patients with invasive breast cancer (BC) and clinically negative lymph nodes (LNs). It successfully replaced the more invasive and morbid axillary lymph node dissection (ALND). The actual standard for SLNB is the radioisotope (RI) with or without blue dye (BD) technique. Because of several drawbacks reported in worldwide experiences, new techniques have been developed in the last years: indocyanine green (ICG) fluorescence, superparamagnetic iron oxide (SPIO) nanoparticles and contrast-enhanced ultrasound (CEUS) using microbubbles. Whilst each technique has its own advantages/disadvantages they are increasing their efficacy and are candidate to represent a new standard for SLNB in next future. This is a comprehensive review of current limitations of conventional techniques besides the improvements and innovations of new methods which, anyway, need future randomized controlled trials to be fully validated.

Keywords: Breast cancer (BC); sentinel node biopsy; indocyanine green (ICG); superparamagnetic iron oxyde; microbubble

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Introduction

According to sentinel lymph node (SLN) hypothesis, tumor cells migrate in an orderly manner from a primary tumour, metastasizing to one or a few LNs before involving others. Therefore, SLN is defined as the first LN that receive lymphatic drainage from the primary tumour, if the tumour has spread. The advent of percutaneous lymphoscintigraphy allowed Robinson *et al.* identify lymphatic drainage patterns of melanomas and the development of SLNB technique through intraoperative lymphatic mapping (1).

Locoregional spread of breast cancer (BC) occurs mainly through the lymphatic system. SLN status accurately predicts the status of the other LNs and is important to establish staging and prognostic outcomes of BC.

SLNB, as ideal nodal staging method for BC, was introduced by Krag *et al.* and Giuliano *et al.* in 1993 and 1994 (2,3). Krag *et al.* described and developed the gamma probe localization of SLN with RI; Giuliano *et al.* described the SNB using BD alone as a procedure technically feasible, safe, and highly accurate to stage regional LNs in BC (4). SNB has since become the new standard of care for axillary staging in clinically and radiologically node-negative BC (5). Clinical trials demonstrated that in these kinds of patients a properly performed SLNB is equivalent to axillary lymph node dissection (ALND) for staging of axilla (6-8), identifying patients who need further axillary clearance, while sparing others a potentially morbid ALND (9).

This paper is a comprehensive review of the currently used techniques for SLNB with a specific focus on the new innovative ones.

Current standard of care for SLNB—blue dye and/or technetium labelled nanocolloid

The traditional SLNB techniques proposed by Giuliano *et al.* (4) and Krag *et al.* (3) have been developed both as single technique and as dual complementary procedure: the choice is determined by surgeon and institutional preference. Giuliano *et al.* reported a 93% SLN identification rate using BD alone, while Krag *et al.* reported a 82% SLN identification rate using only RI and gamma probe.

James *et al.* (10) accurately described best practices for both of these tracers besides to the main problems linked to them.

Three types of BD have been described: isosulfan blue, methylene blue (MB) and patent blue. Each surgeon should look for the method that works best for his or her practice, even though isosulfan and patent blue seem to cause a higher rate of adverse reactions (11). Indeed one of the major problem of BD is a possible adverse reaction up to an anaphylaxis. In case of isosulfan blue it has been reported in 0.7% to 1.1% of cases (12,13). MB has a reduced risk of anaphylaxis but showed side effects as well (14,15): skin necrosis and induration with associated pain, up to pulmonary edema and serotonin syndrome in patients who take serotonergic medications (16). To reduce the potential side effects, the BD are routinely diluted with normal saline (practices vary from 1:1 to 1:7 dilution) (17). Patient should be screened for known allergies and routine prophylaxis is not suggested as a standard but addressed to high risk cases. The injection site has been widely discussed: peripherically to the tumor, in correspondence of the palpable edge of the biopsy cavity, in the periareolar site or into the subareolar plexus. Chagpar *et al.* showed that subareolar and periareolar injection produced higher SLN identification rates than peritumoral injection (18). Even though some authors claim that MB diffuses more rapidly in peripheral tissues, staining a larger portion of the breast with the BD and, to a certain extent, hampering the procedure (19,20), other authors have reported similar accuracy and SLN detection rates (DRs) with MB and with patent blue (21). MB is also much more readily available in different hospitals and less expensive than the others (the cost of MB may represent as little as 3% of the cost of patent blue or isosulfan blue) (22). Another advantage of MB is the possibility to be used during pregnancy (23). In a recent prospective randomized trial MB performed as well as patent blue in identifying SLN in BC (24). MB has so become a standard widely used in USA. Krikanova *et al.* reported a 94.6% SLN identification rate using BD alone making this technique very attractive in high volume units without nuclear medicine facilities or where these facilities are available but when added cost and logistical issues regarding transport of tracer are problematic (25).

The most used RI tracer is the Technetium 99m (^{99m}Tc), ^{99m}Tc-sulfur colloid in USA and ^{99m} Tc-nanocolloid human serum albumin in Europe. It can be injected before surgery around the tumor, intradermally or into the subareolar plexus. Intradermal injection of radiocolloid appears to be superior to subdermal injection (26-28). Recently Berrocal *et al.* described the intraoperative injection of ^{99m}Tc in a large series of patients: they reported this method as convenient, effective, safe, and comfortable for the patient with a SLN DR that was essentially 100% (29). A handheld scintillation counter (gamma probe) is used to guide the surgeon to the labeled LNs. LNs are removed following the "10% rule" (all LNs with counts >10% of *ex vivo* count of the most radioactive node should be removed) (30).

The use of RI creates logistical challenges for hospitals, including the handling and disposal of isotopes, training of staff and legislative requirements. The 6h half-life of the isotope restricts scheduling of surgery because the injection is done by the nuclear medicine department. Additionally, patients might express reluctance to being exposed to radiation and not all of them (mainly the frail patients) have the possibility to reach an hospital with access to RIs (31). These factors have limited the uptake of SLNB worldwide for hospitals without access to RIs (29). Growing interest is arising in development of new and possibly more effective radio-tracers. New ^{99m}Tc-tilmanocept (^{99m}Tc-TM) showed similar SLN uptake to ^{99m}Tc–sulfur colloid but with a rapid injection site clearance, high SLN extraction, low distal node accumulation and no significant pain associated with injection (32). In comparison to ^{99m} Tc-nanocolloid human serum albumin used in Europe showed higher localization rate and degree of localization. ^{99m}Tc-TM has no contraindications, and no serious adverse or hypersensitivity reactions were reported (33). Another interesting radiotracer has been developed by Li *et al.*: ^{99m}Tc-rituximab. It contains ^{99m}Tc-labeled monoclonal antibody targeting CD20 abundantly expressed on the surface of B cells in LNs. The advantage is its uniform molecular weight and molecular size so it will not escape easily from SLNs to the second-echelon LNs achieving a clear SLN imaging in patients and high success rate of lymphoscintigraphy. The sensitivity, specificity, and accuracy of SLNB was 97.40%, 100% and 98%, respectively; the FN rate was 2.60%, which is better than that of other studies (FN rate ranging from 6% to 10%); moreover, its feasibility, safety, and effectiveness have been confirmed by clinical SLNB application with large samples (34). Recently İlem-Özdemir *et al.* prepared a new RI for SLN mapping which chemically combined a RI and a BD, the ^{99m}Tc-isosulfan blue, to be injected as a single dose. They examined its effectiveness in mapping LNs in rats with promising results (35).

Although excellent results are reported in single-institution series using either radioactive colloid or BD (4,36,37) combined use of both tracers appears to be complementary, minimizing the FN rate in most (17,18,38) but not all, studies (39). Motomura *et al.* demonstrated that the combination of BD and RI is superior to dye alone for SLNB in terms of identification rate (95% *vs.* 84%) and sensitivity (100% for the combination) (40).

Therefore, the actual gold standard for SLNB is the dual tracer technique which assures higher SLN identification rates and lower FN rates (41,42). It is particularly indicated for surgeons with limited experience and in cases where misidentification and FN rates are known to be higher (neoadjuvant therapy, prior breast/axillary surgery, obese patients and when the use of BD or radioactive colloid alone fails to produce a signal in the axilla) (43,44). In the dual technique the BD helps with localization post-incision, and LNs that are radioactive, blue, or both are recognised as SLNs (45).

Lymphoscintigraphy is not suggested to be used as routine but only in the cases at higher risk of failing the SLN identification (i.e., previous breast or axillary surgery) (46).

In a meta-analysis of more than 8,000 patients from a systematic review of 69 trials about SLNB, the identification rate of these nodes was 96%, with a FN rate of 7.3% (range 0% to 29%) (47). The identification rate with the dual technique was 97% in the AMAROS trial among 1953 patients with an operable invasive T1-2 BC without clinical suspect regional LNs (48), and 99.1% in patients in the SENTINA trial with advanced disease undergoing SLNB before commencing neoadjuvant chemotherapy (49). In the ALMANAC trial, the dual technique identified SLNs in 96% of patients, but only 85.6% with RI or BD alone (50). In the NSABP B-32 trial, the SLNs were identified in more than 97% of patients (51). Most of the SLNs were both hot and blue (65%), while 24% were hot only, 5% were blue only, and 3.9% were neither hot nor blue, but palpably abnormal (this can be explained since gross tumor involvement interferes with the uptake of both radiocolloid and BD, and lymph flow is diverted to a node other than the true SLN. That's why all axillary palpably abnormal LNs should always be removed). Removal of two SLNs rather than one almost halved the FN rate: in fact the FN rate in this study was 9.8% and was related to the number of SLNs removed: it was 17.7% when only one node was removed, 10% for two nodes, 6.9% for three nodes, and 5.5% for four nodes. This trial also showed no significant difference in overall and disease-free survival between those patients undergoing SLNB followed by automatic ALND and those undergoing SLNB followed by ALND only if the SLNs were identified as positive (6). In a systematic review by the American Society of Clinical Oncology, the use of both BD and RI was associated with an almost significant trend toward fewer FN results (7% *vs.* 9.9%) (42).

James *et al.* (10) particularly stressed the importance of surgeon experience in the accuracy of SLN identification. This was also confirmed by a recent multicenter trial (52). After five training cases, the success rates for individual surgeons identifying a SLN ranged from 79% to 98%. Furthermore, Cox *et al.* showed that surgeons who performed more than six SLNBs per month had lower failure rates (53). Proper surgical technique in SLNB influences outcomes and minimizes the risk of understaging and undertreating patients (10).

The constraints of the existing combined SLNB technique have led to the development of alternative methods which will be the object of the following description and review.

New techniques and future perspectives in SLNB

In recent years new techniques for SLNB have been successfully developed. They use innovative tracers such as indocyanine green (ICG), superparamagnetic iron oxide (SPIO), and microbubbles. Whilst each technique has its own advantages/disadvantages (*Figure 1*), they have shown promising but variable results between studies, small patient numbers and short patient follow-up (45). So they have to be considered still investigational until there is a final evidence that they are accurate in SLNB with a low FN rate.

Indocyanine green (ICG)

ICG is a FDA approved, low molecular weight organic molecule, which fluoresces in the near infrared (NIR) part of the spectrum where tissue absorption of light is minimal and has a well-established safety profile. ICG was initially

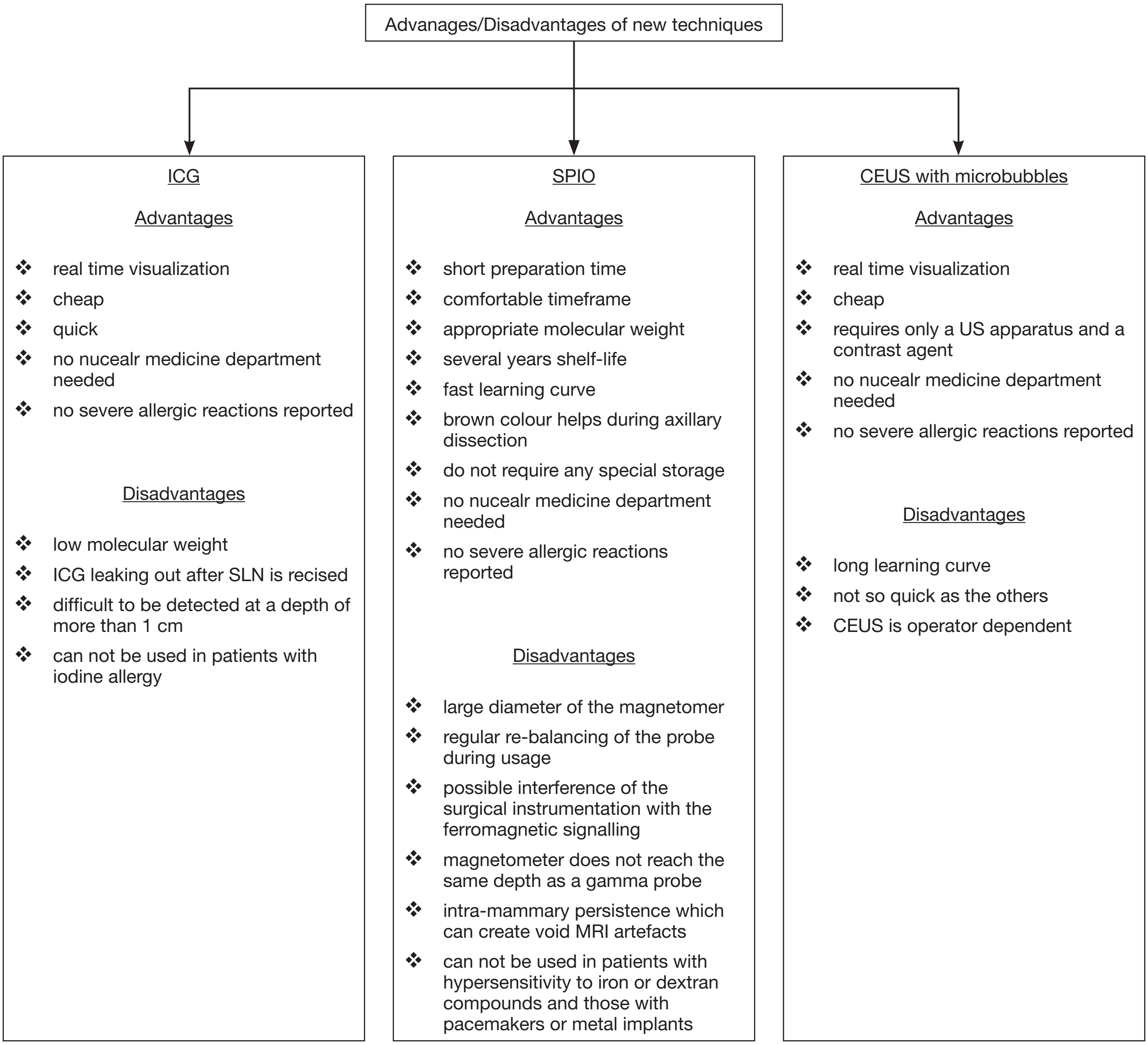

Figure 1 Advantages/disadvantages of new SLNB techniques in BC. ICG, Indocyanine green; SPIO, superparamagnetic iron oxide; CEUS, contrast enhanced ultrasound; SLN, sentinel lymph node; US, ultrasound; SLNB, sentinel lymph node biopsy; BC, breast cancer.

advanced as a visible dye marker in the detection of SLNs, and as such had comparable success to conventional dyes (74% DR in BC) (54). With improvements to image capturing technology (such as sensitive infrared cameras, the photodynamic eye—PDE or the newer Fluorescence Assisted Resection and Exploration—FLARE imaging system), the use of ICG as a NIR fluorophore improved the DR to approximately 95% (55). The fluorescence signal is captured by the PDE using a light-emitting diode producing light at a wavelength of 760 nm in its active state. The detector is a charge-coupled device camera that filters out wavelengths below 820 nm. This NIR fluorescence imaging system visualizes subcutaneous lymphatic flow in real time, as a real time lymphography, and navigates the surgeon to enable an orderly and sequential dissection of SLN.

ICG is injected directly into the breast. The injection site is hugely variable according to the different reported experiences. The majority of studies use periareolar or retroareolar subdermal injection, even though ultrasound-guided injection of ICG near to and surrounding ultrasound-identified SLN has been reported (45). Samorani *et al.* described a protocol of subareolar injection for multicentric BC and intradermal injection for unicentric BC (56). The injected quantity may depend on the surgeon preferences, the breast volume and the BMI of the patient. A recent meta-analysis showed as ICG injection with reduced concentration (<5 mg/mL) and larger volume (≥2 mL) may increase sensitivity and DR (57). A quick breast massage should follow the injection. The time between the injection and the incision varies but it should happen by 10 min. The SLNs are then localized using a fluorescent imaging system (56-58): the tracer progress is followed through the lymphatic ducts to the SLN using an excitation illumination system in combination with a high sensitivity camera, which detects the emitted fluorescence. The axillary skin incision should be made only when the tracer has visibly reached the axilla, not before, otherwise, by interrupting the vessels too soon, the detection of SLNs becomes much more difficult (56). ICG fluorescence is scattered by superficial tissues and is difficult to be detected at a depth of more than 1 cm. Thus caution is required when searching for SLNs at lower depths particularly in obese patients.

A major advantage of ICG is that it enables real time visualization of lymph flows from the breast to the axilla. Thus, SLNs can be identified and resected more rapidly and easily, especially in cases with multiple lymph drainage pathways, where ICG can detect multiple SLNs. ICG is reported to be much cheaper compared with a radiotracer. The fluorescence imaging device is also cheaper than a gamma probe. Five milligrams of ICG costs about $1.50, while a radiotracer is about $200 in Japan (59). Therefore, as a result of the lower cost, patients in developing countries may also benefit from ICG tracer. Moreover, the involvement of a nuclear medicine department is not necessary and there is no exposition to radiations. On the other hand, it has been observed a higher number of SLNs identified with ICG, probability due to the low molecular weight which translate into rapid migration in the lymphatics spreading beyond the SLN to secondary draining LNs (55). In their review Ahmed *et al.* (45) reported a mean number of nodes excised between 3 and 5.4 for ICG and between 1 and 2.4 for BD. ICG probably travels to higher echelon nodes than BD does, resulting in unnecessarily excessive dissection and removal of nodes.

Furthermore, when the first SLN is resected, lymph vessels are cut and ICG leaks out. The leaked ICG spreads to the surgical field, making it difficult to detect another fluorescent node. This problem can be solved by reducing the amount of ICG. Lastly ICG cannot be used in patients with iodine allergy, because it contains iodine.

Since ICG fluorescence is visualized by collecting NIR rays in dimmed light conditions, recently Toh *et al.* developed a new promising ICG imaging system called HperEye, which allows the transcutaneous visualization of lymphatic vessels under normal light conditions, thus facilitating the identification and detection of SLNs without affecting the surgical procedure, together with a high sensitivity and specificity (60).

Ahmed *et al.* found that ICG was significantly better than BD in terms of improved SLN identification, while there was no statistically significant difference between ICG and RI although almost all showed SLN identification to be higher with ICG. No difference was noticed also between ICG and the dual technique. They reported a SLN identification rate between 93.1% and 100% (in 11/15 studies) of patients (45).

Increasing clinical results for the ICG method support a higher SLN DR (99–100%) compared with the use of BD (61,62). Moreover, several clinical trials demonstrated that ICG method is safe and achieves a high SLN DR that is comparable or superior to the RI method (56,63-65). Sugie *et al.* reported a similar overall DR of SLNs between ICG and RI with a significant improvement achieved by the combination of methods compared with RI alone (66). Tong *et al.* showed that a combined ICG and BD tracer technique was superior to the use of BD alone for identifying SLNs with higher sensitivity, and for predicting axillary LN status in patients with BC; in addition, the combined technique had reduced FN results (67). Similar results were reported by Hirano *et al.* in a large series of patients (59). Samorani *et al.* reported their experience in the use of ICG only for SLNB and they demonstrated that there are no advantages in the association of ICG with BD, as the use of ICG only, once the technique is mastered, allows, always and in every case, the removal of the SLN with a 100% DR (68). On the other hand, Ji *et al.* affirmed that the efficiency and sensitivity of SLNB can be improved by combining ICG with BD since the use of blue tracer significantly decreased the average time to detect each SLN, and it increased the number of SLNs identified (69).

To confirm the importance of this upcoming technique a recent meta-analysis by Zhang *et al.* reported that ICG guided SLNB had a 98% DR; the pooled sensitivity (0.92) and specificity (1) were relatively high and the FN rate was relatively low (8%) going down to 4% when when ICG was combined with BD; in the presence of metastases, the diagnostic performance of this method was good, with relatively high sensitivity and very high specificity (70).

The last published meta-analysis on this topic of 12 non-randomized comparative studies, reported that ICG was equal to or better than RI in localizing SLNs and tumor-positive SLNs. These results confirm that the ICG fluorescence method is a useful alternative to the standard RI method for SLNB (71).

No severe adverse events directly related to the ICG dye have been reported. Recent improvements in this technique, combined with its good safety profile, made the use of ICG to find SLNs an attractive and promising alternative for SLNB in BC. However, a good standardisation of the technique between studies is low, which makes it difficult to reliably draw final conclusions. This shows how this technique is still in development with significant refinement necessary (45).

Superparamagnetic iron oxide (SPIO)

SPIO is a non-invasive magnetic tracer for SLNB detected by a handheld magnetometer. The SentiMAG multicentre trial (72) was the first trial that evaluated this new magnetic technique for SLNB against the standard one. When injected subcutaneously, SPIO moves into SLNs within minutes, and iron deposition is seen predominantly within sinuses and in macrophages. In the event of metastatic involvement of the node, SPIOs are seen to deposit within uninvolved areas of the node only (73). The nodes can be visualized on magnetic resonance imaging (MRI) and at operation are often colored brown or black (74). The SentiMAG trial group developed 2 CE marked devices: an injectable magnetic tracer (Sienna**+**) and a handheld magnetometer (SentiMag) that generates an alternating magnetic field which temporarily magnetizes the SPIO and senses the particles' magnetic response. The particle diameter of Sienna+, including its organic coating, is 60 nm. It is ideally suited for SLNB since this diameter enables the SLNs to selectively filter out the particles and is similar to the particle size of standard RI tracers. The diameter of the magnetometer is slightly larger (6 mm) than that of the gamma probe. The magnetic technique was well standardized. A 5 mL periareolar subcutaneous injection was administered, consisting of 2 mL of magnetic tracer (Sienna**+**) diluted with 3 mL of normal saline. This was injected intraoperatively (after induction of anesthesia) followed by a 5 min massage. The median Sienna+ migration time before surgery was 20 min. The surgeon used the handheld magnetometer for the SLN localization. All SLNs detected intraoperatively by using the handheld magnetometer or gamma probe or nodes that were blue or black, were excised. All metal retractors were removed from the surgical field while the magnetometer was used. Excision of nodes identified by magnetometer followed the same "10% rule" used for the gamma probe. Any palpable nodes were also removed. They reported no difference in identification rate (95% with the standard technique and 94.4% with the magnetic technique) and no difference in the average number of LN removed (1.9 *vs.* 2.0 per patient) (72).

Similar results were achieved by the Central-European SentiMag study (75) reporting similar DRs between the standard RI technique and the magnetic one (97.3% *vs.* 98.0%), with a similar average number of removed SLNs per patient and a higher per patient malignancy DR for the SPIO tracer.

In agreement with the previous experiences Rubio *et al.* showed no difference in DR between the standard RI technique and the magnetic one (95.7% *vs.* 98.3%) with a significant higher average number of SLN excised in the SPIO group (76).

The "IMAGINE" Spanish multicentre study reported similar DRs between RI technique and magnetic technique with the *ex-vivo* and intraoperative DRs at the node level slightly higher for the magnetic technique. The SPIO tracer also showed a higher per patient malignancy DR (77).

The French Sentimag Feasibility Trial evaluated the magnetic technique in comparison to the standard technique (RI with or without BD) (78). The DR was 97.2% for Sienna+ and 95.4% for the standard technique with more SLNs detected with Sienna+ (97.2% *vs.* 90.2%). Furthermore, the N+ status does not seem to be an obstacle for the magnetic method which shows a tendency to identify more involved SLNs, a trend already described by Thill *et al.* (75).

A meta-analysis of five clinical trials comparing Sienna+ to a standard technique confirmed that Sienna**+** is non-inferior with respect to SLN identification per patient, as well as per SLN. Importantly, non-inferiority was also shown with respect to the ability to accurately identify patients with malignant SLNs (79).

In another recent meta-analysis of seven randomized trials by Zada *et al.*, the SPIO technique was not inferior to the standard technique in identification rate (97.1% *vs.* 96.8%), total LNs retrieved (1.9 *vs.* 1.8 nodes per patient), and FN rate (8.4% *vs.* 10.9%). There was a trend towards a lower FN rate in favour of the magnetic technique, but this was not statistically significant. The total number of LNs retrieved was significantly higher with the magnetic technique. The mean discordance rate between the two techniques was 3.9% (range 1.7% to 6.9%) (80).

The SentiMag technique offers many advantages: surgeon can inject the magnetic tracer directly in the operation room, the preparation time is much shorter than for the RI, provides a very comfortable timeframe and the SPIO tracer is well retained in the "true" SLN. The shelf-life of the magnetic tracer is several years and the learning curve seems to be very fast, especially if compared to the other techniques. The brown color does not seem to be sufficient to make identification alone but can help the surgeon during the axillary dissection. Sienna+ particles do not require any special storage and there are no radiation risks or any of the legislative controls associated with the handling of isotopes, neither for healthcare personnel nor for the patient. On the other hand, the diameter of the magnetometer is quite larger than the RI probe, even though a smaller one has been recently commercialized. Moreover, this technique requires a regular re-balancing of the probe during usage before each signal acquisition. Another technical limitation is a possible interference of the surgical instrumentation with the ferromagnetic signalling. To prevent it, the use of plastic surgical material is encouraged while the measurement with SentiMg is performed. Particular attention should be given to the intra-mammary persistence of Sienna+ which can create void artefacts and could hamper the interpretation of a postoperative breast MRI obscuring important clinical findings. Subareolar injections may have a higher risk of void artefacts than intratumoral or peritumoral injections of small magnetic tracer volumes, because the tumour area is excised (80). In a small study of ten MRI scans performed in six patients, void artifacts were noted at the subareolar injection site in all scans and all artifacts were greater than 5 mm in greatest dimension. Based on current evidence, the magnetic technique should not be used in patients undergoing SLNB before primary chemotherapy (if MRI is used to assess response to treatment), those undergoing breast MRI surveillance (such as BRCA mutation carriers) or those with a mammographically occult tumour at diagnosis, in whom MRI may be required for further assessment or follow-up imaging (81). More research should clarify the patient cohort eligible for the magnetic technique and optimize the amount of magnetic tracer required. Another concern is that the magnetometer does not reach the same depth as a gamma probe, which can have consequences for the identification of deeper nodes, as demonstrated in the recently published MELAMAG Trial (82). Patients with hypersensitivity to iron or dextran compounds and those with pacemakers or metal implants were excluded from the clinical trials and would not be candidates for using Sienna+ for SLN mapping. A dermopigmentation is the most frequent complication reported at a rate up to 20% in correspondence of the injection site, which usually vanished over time (76,78). No severe allergic reactions were reported in any of the trials and meta-analysis reviewed.

All these encouraging results led to consider magnetic technique as feasible, but its performance should now be evaluated in a large randomized non-commercial controlled trial before clinical implementation (80).

SPIO enhanced MRI

Motomura *et al.* evaluated SPIO enhanced MRI for the detection of metastases in SLNs of patients with BC. A node was considered involved if the entire node or a focal area did not show low signal intensity on MRI. The sensitivity, specificity, and overall accuracy of MRI for the diagnosis of SLN metastases were 84%, 91%, and 89%, respectively (74). A more recent update by the same group introduced a SPIO enhanced MRI at 3T (83). They reported much better results: On a patient-by-patient basis, the sensitivity, specificity and accuracy of MRI for the diagnosis of SLN metastases were 100%, 96% and 97%. Authors concluded that SLNB may be avoided in patients with BC who have non-metastatic SLNs on SPIO-MRI.

The SentiMag multicentre trial imaging subprotocol evaluated the use of SPIO MRI for pre-operative localization of SLN and its potential for non-invasive identification of SLNs metastases (84). SPIO contrast agents are known to provide "negative contrast" on T2 weighted MRI. SPIOs are injected interstitially, they are taken up by SLNs, and therefore SPIO MRI can be used as an alternative for pre-operative localization of SLNs which are visualized as a drop of signal intensity. LNs showing inhomogeneous SPIO uptake were classified as metastatic. SPIO-MRI successfully identified SLNs in 91% of patients. One patient had metastatic involvement of four LNs, and this was identified in one node on preoperative MRI. Two false positive cases were reported. The concordance between the number of nodes identified by imaging and during surgery was 55% for both the magnetic and the combined technique. So it seems that SPIO MRI is a feasible technique for preoperative localization of SLNs and, in combination with intraoperative use of a handheld magnetometer, provides an entirely RI-free technique for SLNB. It gives the surgeon a detailed anatomical information on the location of the SLNs, which serves as a detailed surgical roadmap. Moreover, a noninvasive method that accurately diagnoses SLN metastases would prevent patients undergoing unnecessary SLNB. However further optimization of the technique and research with larger series of patients is needed.

Li *et al.* evaluated magnetic resonance lymphography with gadolinium (Gd-MRI) in SLN identification and metastasis detection in patients with BC (85). There was a significant correlation between the SLN numbers found by Gd-MRI and BD methods. Using BD as the gold standard, the sensitivity of Gd-MRL was 95.65% and the FN rate was 4.3% for axillary lymphatic metastasis detection. With heterogeneous enhancement and enhancement defect as the diagnostic criteria, Gd-MRL gave a sensitivity of 89.29% and specificity of 89.66% in discriminating malignant from benign SLNs.

Compared with SPIO-MRL, Gd-MRL is cheaper and more convenient. SPIO is a negative contrast and thus cannot image the lymph vessel. Compared with iopamidol-CT lymphography, Gd-MRL lacks radiation exposure, possibility of anaphylactic shock and nephrotoxic impairment (86). The good results achieved by this technique suggest a potential value in a future clinical practice.

Contrast enhanced ultrasound (CEUS) with microbubbles

This is an innovative technique where microbubble contrast agent, based on the use of dispersion with sulfur hexafluoride gas, is injected intradermally around the areola. Breast lymphatics are then visualized by CEUS and followed to identify and biopsy SLNs (87).

Sever *et al.* tried to standardize a protocol (88). They performed a periareolar intradermal injection of 0.2–0.5 mL phospholipid-stabilised microbubbles containing sulphur hexafluoride gas with a mean diameter of 2.5 µm (Sonovue-Bracco) reconstituted with 2 mL sterile saline. The breast was massaged for 10–30 s and lymphatic channels were visualised immediately on contrast pulse sequencing and followed into the axilla. The transit time from injection to arrival in the axillary nodes was 15–45 s. Areas of contrast accumulation were imaged with greyscale or live dual images to confirm the presence of an architecturally defined LN. The CEUS-identified SLNs were then localized with guidewires before undergoing SLNB with the standard dual technique. It resulted in SLN identification in 89% of patients (89). In a larger series by Cox *et al.* the technique failed to visualise or successfully biopsy 13.3% of patients, identifying positive SLN in 87.7%. There were 22 false-negative results, which meant that sensitivity was 61% (low compared to the dual technique) and specificity 100% (87).

In the review by Ahmed *et al.* the standard dual technique was significantly better than CEUS in terms of SLN identification rate which anyway was between 87.7% and 89% (45).

A recent meta-analysis of five studies reported the SLN identification rate ranging from 9.3% to 55.2%, the sensitivity from 61% to 89 %, the FN rate from 6.6% to 39% and the presence of micro/macrometastases from 1.9% to 64.3% (90).

Better results were achieved by Xie *et al.* who studied CEUS with Sonovue intradermal subareolar injection to localize SLN and the value of enhancing patterns in diagnosing SLN metastases (91). They showed no significant difference in SLN identification rate between CEUS and BD (97% *vs.* 96%). CEUS method detected less SLNs than BD while the positive rates of SLNs identified by CEUS was significantly higher than that by BD. Sensitivity of predicting SLNs metastases by CEUS enhancing pattern was 81.8%, the specificity was 86.2%, and the accuracy was 84.7%. They also described three patterns of enhancement of SLNs and the disomogeneous one was more common in involved SLNs.

In a preliminary clinical trial by Matsuzawa *et al.*, CEUS was performed with a subareolar contrast injection to evaluate the SLN detection and intravenous contrast injection studied at color doppler to evaluate the presence of metastases in SLN, visualizing their microvascularities (92). The peripheral vascularization pattern was more frequently found in metastatic LNs. They used Sonazoid as contrast agent, a perflubutane microbubble that is stabilized using a phospholipid. Their results were very promising: sensitivity 81.8%, specificity 95.2%, positive predictive value 90.0%, negative predictive value 90.9%, accuracy 90.6%.

Dellaportas *et al.* reported their experience with CEUS using totally intravenous contrast injection for a preoperative detection of malignant SLN (93). They showed a negative predictive value of 90% and positive predictive value of 75%. Overall sensitivity was 83.33% and specificity was 84.38%. Multivariate analysis showed that the CEUS outcome was only correlated with the actual final histopathological report on the SLN. All the cases with 3 or more positive LNs were detected preoperatively with CEUS. They observed that involved nodes enhanced heterogeneously, with an early wash-in and wash-out enhancement.

Last meta-analysis published on this topic by Moody *et al.* evaluated whether CEUS-guided core biopsy of SLN could identify metastatic nodes preoperatively reducing the number of surgical SLNBs and whether CEUS SLN identification and localization is a viable alternative to standard lymphatic mapping using RI and BD (94). The SLN identification and localization rate for CEUS-guided skin marking was 70–100% and for CEUS guided-wire localization was 89–97%. Across the four studies that evaluated preoperative CEUS-guided SLNB, pooled sensitivity for identification of nodal metastases was 54% and pooled specificity 100%; the FNR ranged from 8% to 17%.

One advantage of this method is the real-time imaging for SLNs. It requires only a US apparatus and a contrast agent readily available on market so it is cheap and does not need radioactive materials. The patient is not exposed to radiation and the method is not invasive. CEUS guided core biopsy could potentially decrease the number of SLNBs and ALNDs in women with BC. CEUS has been proved to be accurate to identify and localize the SLNs preoperatively with SLN identification rates comparable to standard lymphatic mapping with RI and BD. So CEUS-guided SLN localizations may offer a viable alternative for developing countries that may not have access to nuclear medicine facilities. There are no iodine and proteins in sulfur hexafluoride microbubbles, which prevents patients from allergy. Indeed there have been reported no significant side effects or complications from the injection of microbubbles.

On the other hand, the learning curve for this technique seems to be quite long and the US still remains operator dependent.

Even though it is a promising technique both for SLNs identification and for metastasizing SLNs detection, further studies are necessary to well standardize the method and improve the sensitivity and specificity. Randomized control trials with large series of patients are suggested in order to compare this technique with the current techniques used for SLN detection.

Conclusions

SLNB is still a stronghold in the management of early BC since the status of the axillary LNs remains one of the most important prognostic factors. Although the standard dual technique has reached a good level of standardization and accuracy it is burdened by several drawbacks. The use of RI creates logistical challenges for hospitals, legislative requirements, exposition to radiations both for the patient and for the healthcare staff. Moreover, the 6 h half-life of the isotope restricts scheduling of surgery because the injection is done by the nuclear medicine department. The development of new techniques in SLNB together with improvement in surgical strategies are clouding the traditional methods. Incoming experiences in endoscopic SLNB and ALND have been described, reporting low complication rates, better cosmetic results and similar efficacy to the traditional open axillary surgery (95). The new SLNB techniques seem to be safe, feasible and have shown very high improvements in accuracy, sensitivity and specificity in last years. It would be time to develop new comparative studies between the new techniques themselves to better clarify their value and efficacy. Anyway, all the last evidences show similar results or better than the traditional ones. Surgeon can be independent from the nuclear medicine department and can manage the operating independently. However, it should be well clarified how easy the availability of the new devices is and their relative cost.

Since the new methods have now achieved a high qualitative standard and seem to be totally competitive with the traditional ones, randomized controlled trials are needed to assess their outcomes against the standard dual technique and be fully approved as new standard of care for SLNB.

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Footnote

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