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REVIEW ARTICLE

Sentinel Lymph Node Identification Using Contrast Lymphosonography: A Systematic Review

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Abstract: The sentinel lymph node (SLN) concept hypothesizes that metastatic cancer cells will spread through the lymphatic system to the SLN being the first one in the lymphatic chain to receive the metastatic cells, indicating that if the SLN is free of cancer cells the rest of the lymphatic chain is also without metastatic disease. Diagnostic ultrasound imaging (US) has been used to evaluate lymph nodes (LN) to determine level of suspicion and to guide LN biopsies. However, conventional US cannot be used for lymphatic mapping, which requires administration of a tracer. This has been changed with the use of contrast-enhanced US (CEUS) to detect lymphatic channels and SLNs after subcutaneous injections of microbubble-based US contrast agents (UCAs). The aim of this review is to examine the clinical evidence on the role of subcutaneous injection of UCA, known as lymphosonography, to be used as preoperative identification of SLNs in patients with breast and other cancers.

Key words: Contrast-enhanced ultrasound; Sentinel lymph node; Breast cancer; Lymphatic tracer; Ultrasound contrast agent; Lymphosonography; Lymphatic mapping

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The sentinel lymph node (SLN) is the first lymph node (LN) in the lymphatic chain draining a particular location. The SLN concept theorizes that in solid tumors with regional metastatic potential, the metastatic cells will spread through the lymphatic system to the SLN being the first one to receive the metastatic cells [1-5]. Therefore, if the SLN is deemed to be free of metastatic cancer cells the remaining regional LNs are also considered to be negative for metastatic disease, conversely if the SLN contains infiltrating metastatic cells then all the remaining LNs in that lymphatic drainage pathway are considered to be positive for metastatic disease [1,3,4]. The determination of the presence and extent of regional LN involvement is used to guide treatment selection and remains the most powerful predictor of recurrence and survival [6-11]. At the time of the cancer diagnosis the regional lymphatic chain and LNs are evaluated clinically and/or radiologically. Any LN considered to be suspicious will undergo core-biopsy to determine diagnosis, since histopathologic tissue analysis is currently the only way to accurately determine LN metastatic involvement [12].

However, the majority of patients will have no suspicious LN findings at the time of diagnosis, which leaves surgical excision and histopathologic analysis of the regional LNs the only way to determine the final stage of disease. Accurate assessment of potential LN involvement is essential to limit the extent of LN removal in order to only remove the LNs direct connected with the tumor area and minimize the anatomical disruption caused by an extensive axillary LN

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removal, which can result in lymphedema, nerve injury, shoulder dysfunction, and other complications that may compromise functionality and quality of life [12,13].

The mapping of the lymphatic system, currently, is performed after injection of blue dye, indocyanine green (ICG) and/or a radioactive tracer followed by surgical excision [3,14-18]. However, there are several limitations with these approaches. Radioactive tracers can give an indication of the position of the SLN using hand-held radiation detection probe, however, without any imaging component and the additional use of radiation [16,19-21]. There is also a time sensitive component to this procedure, since by the time of the surgical excision the small size of the radioactive colloid means that the isotopes may have passed through the SLN entering secondary and/or tertiary LNs, risking unnecessary resection of LNs [16,19-21]. The injection of blue dye suffers from the same issue, where the velocity at which the blue dye proceeds through the lymphatic system determine that the injection of blue dye has to be done at the beginning of the surgical excision [16,19-21]. The main issue with the injection of ICG is that the tissue penetration capacity of near infrared (NIR) fluorescence is inferior to that of gamma rays leading to worse overall performance in patients, especially in those with a larger body habitus [14,22]. Also, the presence of macrometastasis can limit the diffusion of ICG from the LCs to the LNs [14,23-25].

Conventional diagnostic ultrasound (US) imaging modes such as grayscale, color Doppler and power Doppler (combined or individually) are part of clinical patient care to determine the level of suspicious of a LN for malignancy [26-29]. However, US on its own cannot be used for lymphatic mapping, since mapping requires administration of a tracer. This limitation was conquered when reports on the use of contrast-enhanced US (CEUS) to detect LCs and SLNs after subdermal injections of microbubble-based US contrast agents (UCAs) in animal models and clinical trials (termed "lymphosonography") were produced [20,21,30-34]. The development of the lymphosonography technique, which uses CEUS to evaluate LN, addressed the limitations of the currently used lymphatic mapping techniques. Pre-clinical studies using a Sinclair swine model with naturally occurring melanoma tumors showed that the UCA used as lymphatic tracer in lymphosonography stay restricted to the SLNs and does not progress further to the echelon LNs in the lymphatic system [20,21,30,35].

The aim of this review was to examine the clinical evidence on the role of subcutaneous injection of UCA, known as lymphosonography, to be used as preoperative identification of SLNs in patients with breast cancer compared to pathology and/or standard of care lymphatic mapping methods. This review also included the few clinical studies using lymphosonography for other types of cancer.

Application in Breast Cancer

The translation to clinical trials with human subjects was primarily focused on the use of this technique to evaluate the presence of SLNs in humans with breast cancer. The overall majority of clinical trials on the subject of lymphosonography evaluated the identification of SLN in patients with breast cancer. That demonstrates the great impact that correct SLN identification has for this particular type of cancer and how there are still room for improvement in the identification of the correct SLNs that are draining the tumor. The overall majority of clinical trials on the subject of lymphosonography evaluated the identification of SLN in patients with breast cancer [9,15,32-34,36-56]. These studies demonstrates the great impact for improvement in the identification of the correct SLNs that are draining the tumor.

A group from the UK was one of the pioneers in the clinical use of lymphosonography in patients diagnosed with breast cancer and their experience was published in six peer-reviewed papers [33,34,36-39]. The patient population that underwent lymphosonography were patients diagnosed with breast cancer that had negative axillary findings in clinical as well as imaging. The UCA SonoVue (Bracco, Italy), in a dosage of 0.2-0.5 mL was injected intra-dermally peri-areolar of the breast. The injected region was massaged for 10-30 seconds after injection and lymphosonography was performed. The SLNs identified using CEUS underwent biopsy procedures (core-biopsy or fine-needle aspiration). Only one SLN was biopsied for each patient. The patient then underwent surgical excision with SLN biopsy (SLNB) or axillary nodal dissection based on the pathology results from the CEUS guided biopsy. The CEUS biopsy findings were compared with the final surgical pathology results acquired by using the standard lymphatic mapping consisting of radioactive tracer and blue dye together. Cox et al. [37] published in 2018 a paper with the largest dataset of patients from this group which evaluated the results from 1,906 patients studied at four Medical Centers under varying protocols. SLNs were identified in 1,653 patients with successful biopsy being performed in 1,452 patients, which translates to a sensitivity of 47.8% and a specificity of 98.5% for the successfully SLN biopsied using lymphosonography [37]. The comparison between the SLNs identified with CEUS and the SLNs identified with the dual lymphatic mapping standard of care was achieved by clips placed during the CEUS guided biopsy and identified by pathology

after the surgical excision of the SLNs identified by the dual lymphatic mapping [33,34,36-39]. Table 1 shows a summary of this group's studies.

The combined use of two lymphatic mapping approaches prior to the surgical excision of SLNs in patients with breast cancer is the standard of care at many Medical Centers (Consensus Guideline on Axillary Management for Patients With In-Situ and Invasive Breast Cancer: A Concise Overview, Am Soc Breast Surg, March 14, 2022). Injections of both blue dye and radioactive tracer prior to the surgical excision for SLNs identification has been used for comparison to SLN identification by lymphosonography in several studies [6,7,33,34,36-41,49,53,56-59]. Kim and colleagues [7] reported on studies that used only blue dye, only radioactive tracer and also on studies that used both blue dye and radioactive tracer in their meta-analysis. Results showed that successfully mapping SLNs across these 3 groups of patients were achieved in 83.1%, 89.2%, and 91.9% of studies, respectively ($P = 0.007$), with the false negative rates being lower for the group of studies that used both blue dye and radioactive tracer. (10.9% only blue dye, 8.8% only radioactive tracer, 7.0% both blue dye and radioactive tracer ($P = 0.047$) [7].

Table 1 Summary of Sever's group studies on the use of lymphosonography in patients with breast cancer

Author	Year	Patients (*n*)	Results
Cox	2013	371	SLNs identified in 333 patients, biopsy was successful in 295 patients. Sensitivity: 65%, specificity: 100%, PPV 100% and NPV 95%.
Cox	2016	903	SLNs identified in 605 patients, biopsy was successful in 555 patients.
Cox	2018	1,906	SLNs identified in 1,653 patients, biopsy was successful in 1,452 patients. Sensitivity: 47.8%, specificity: 98.5%, PPV 91.6% and NPV 86.2%.
Moody	2020	240	SLNs identified in 186 patients, biopsy was successful in 163 patients. Sensitivity: 65%, specificity: 100%, PPV 100%, NPV 82.84% and accuracy: 85.89%.
Sever	2011	80	SLNs identified in 71 patients, biopsy was successful in 62 patients. Sensitivity: 89%.
Sever	2012	136	SLNs identified in 132 patients, biopsy was successful in 126 patients. Sensitivity: 55.77%, specificity: 100%, PPV 100% and NPV 92%.

Our group conducted a clinical trial with the objective to evaluate the efficacy of lymphosonography to identify SLNs in breast cancer patients undergoing surgical excision with the use of blue dye and radioactive tracer for guiding SLN excision and the results from lymphosonography compared with blue dye and radioactive tracer using pathology results as the reference standard [56]. A total of 86 subjects were enrolled and 79 completed the study and received subcutaneous 1.0 mL injections of the UCA Sonazoid (GE Healthcare, USA) around the tumor. Contrast-enhanced lymphosonography was used to identify SLNs associated with the tumor. The subjects then underwent surgical excision as part of their standard-of-care. The surgically excised SLNs were classified as positive or negative for presence of blue dye, radioactive tracer and UCA, and sent for pathology. A total of 252 SLNs were excised, 158 positives for blue dye, 222 positives for radioactive tracer and 223 positives for UCA. The comparison to blue dye showed accuracy of 96.2% for radioactive tracer and 99.4% for lymphosonography ($P > 0.15$); and in the comparison to radioactive tracer, blue dye showed accuracy of 68.5%, while lymphosonography achieved 86.5% ($P < 0.0001$). Of 252 SLNs excised, 34 were determined malignant by pathology; 18 positives for blue dye (detection rate 53%), 23 positives for radioactive tracer (detection rate 68%) and 34 positives for UCA (detection rate 100%; $P < 0.0001$) [56]. Figure 1 shows an example of a SLN in a breast cancer study case from our group's clinical trial.

Nonetheless, there were several studies that used only blue dye as the standard of care lymphatic tracer [9,43,45,47,50,52,58,60]. Although, lymphatic mapping using the dual combination of both blue dye and radioactive tracer is the most accepted method, radioactive tracers are expensive, require pre-operative preparation and present logistic challenges regarding the handling of radioactive material [9]. Alternately, blue dye is less expensive and requires no specific apparatus and therefore many institutions still use only blue dye to perform lymphatic mapping in clinical practice [9,61]. However, the use of blue dye alone has been shown to result in higher false-negative rates [9,62]. Li at al. [43] compared lymphosonography used for SLNs CEUS-guided biopsy with lymphatic mapping using blue dye and the results showed an identification rate of 98.2% and a coincidence rate of 95.8% for lymphosonography.

Application in Other Types of Cancers

There are only a small number of clinical trials employing lymphosonography to evaluate SLN identification in patients with cancers located in other organs than the breast [63-67]. All these studies can be considered to be pilot studies, since all of them involved no more than 30 patients [63-67]. Table 2 shows a summary of the results from the studies using lymphosonography for SLN identification in patients with other types of cancers.

Our group conducted a pilot study with the objective to compare the performance of contrast-enhanced endoscopic ultrasound (CE-EUS) guided fine needle aspiration (CE-EUS-FNA) with the EUS-FNA for lymph node (LN) staging in esophageal cancer using pathology as the reference standard [67]. Thirty subjects with esophageal cancer scheduled to EUS-FNA staging underwent EUS and CE-EUS with FNA using a curvilinear endoscope ultrasound probe (Hitachi, Japan). All LNs identified by standard EUS were first noted, then the UCA Sonazoid was administered peri-tumorally and all enhanced LNs were recorded. FNA was performed on LNs considered suspicious by EUS alone as well as LNs enhanced on CE-EUS, the performance from both ultrasound imaging modalities was compared using FNA cytology as reference standard. A total of 132 LNs were detected with EUS, of those 59 showed enhancement on CE-EUS. Fifty-three LNs underwent FNA, 22 LNs were determined to be malignant by pathology with 10 being considered suspicious by EUS and the other 12 LNs underwent FNA only due to CE-EUS enhancement. CE-EUS showed enhancement in 19 of the 22 malignant LNs. The rate of metastatic node identification from EUS was 45% (10/22) and it was 86% (19/22; $P = 0.008$) for CE-EUS [67]. Figure 2 shows an example of a SLN in an esophageal cancer study case from our clinical trial.

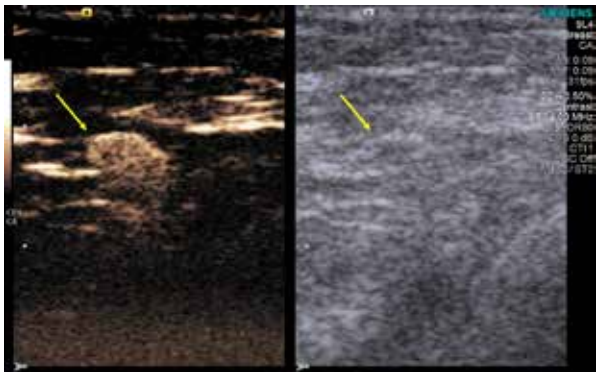

Figure 1 Example of a breast cancer study case. The subject is a 76 years-old female patient diagnosed with an invasive ductal carcinoma located on the left breast at 2 o'clock position measuring 1.0 cm. After the surgical excision, the SLN was sent to pathology, which determine to be negative for metastatic disease. The SLN was positive for the presence of blue dye, radioactive tracer and UCA at the time of the excision. The figure shows a dual-image CEUS and B-mode of the SLN (arrow).

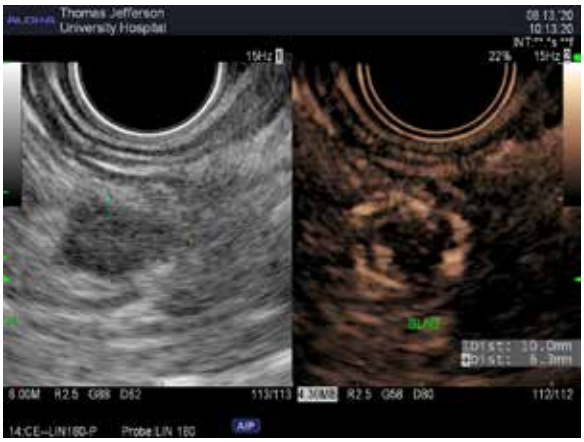

Figure 2 Example of a SLN in an esophageal cancer study case. The subject is a 72 years-old male patient diagnosed with an esophageal adenocarcinoma located on the lower portion of the esophagus. The figure shows a dual-image B-mode and CEUS of the SLN (arrow), with SLN enhancement seen in the CEUS image.

Wakisaka et al. [63] conducted a pilot study in 10 patients with a diagnosis of oral or oropharyngeal cancer who clinically presented with a lesion in the N0 category. The UCA Sonazoid was injected peri-tumorally in 9 out of the 10 patients (in one case the UCA was injected intra-tumorally), with SLNs being identified in 8 out of the 10 patients.

Another pilot study for oral cancer was conducted by Gvetadze et al. [65] in 12 patients with stage T1–2cN0 squamous cell carcinoma (SCC) of the tongue. A total of 15 SLN were found in 11 cases (1.4 SLN/patient). Lymphosonography identified SLNs in 11 of the 12 patients for an identification rate of 91.7%.

Wei et al. [64] conducted a pilot study in 24 patients with papillary thyroid carcinoma undergoing cervical lymph nodes staging. The UCA Sonazoid was injected into the superficial thyroid parenchyma in front of the tumor to identify draining SLNs. The study evaluated the value of the combination of lymphatic-CEUS (LCEUS) and intravenous-CEUS (IVCEUS) to identify cervical lymph node metastasis (CLNM) from papillary thyroid carcinoma (PTC). Benign LNs displayed a complete bright ring (100%) and homogeneous perfusion (88.9%) on LCEUS, while displaying centrifugal perfusion (66.7%) and homogenous enhancement (88.9%) on IVCEUS. Perfusion defects (94.9%) and interruption of the bright ring (71.8%) were the two characteristic LCEUS signs for diagnosing CLNM. On IVCEUS, CLNM appeared as centripetal perfusion (59.0%) and heterogeneous enhancement (59.0%). LCEUS had more value (AUC = 0.850) in diagnosing CLNM than IVCEUS (AUC = 0.692) and routine US (AUC = 0.581). The combination of LCEUS and IVCEUS has the highest diagnostic value (AUC = 0.863). The results showed

Table 2 Summary of studies on the use of lymphosonography in other types of cancers

Author Year Country	Patient (*N*) Cancer type	UCA/Dose Injection site	Comparison	Results
Liu, et al. 2022 USA	*N* = 30 Esophageal Adenocarcinoma and squamous cell carcinoma	Sonazoid/1.0 ml Peritumoral	FNA cytology	CE-EUS showed enhancement in 19 of the 22 malignant LNs. The rate of metastatic node identification from EUS was 45% (10/22) and it was 86% (19/22; *P* = 0.008) for CE-EUS.
Wakisaka et al. 2019 Japan	*N* = 10 Oral Squamous cell and mucinous epidermoid carcinomas	Sonazoid/2.0 ml Peritumoral	Surgical pathology	SLNs were successfully detected in 8 out of 10 cases.
Wei et al. 2021 China	*N* = 24 Thyroid Papillary carcinoma	Sonazoid/0.1 ml At front of the tumor	FNA cytology	To evaluated lymphatic-CEUS (LCEUS) and intravenous-CEUS (IVCEUS) to identify cervical node metastasis (CLNM). LCEUS had more value (AUC = 0.850) in diagnosing CLNM than IVCEUS (AUC = 0.692) and routine US (AUC = 0.581).
Gvetadze et al. 2017 China	*N* = 12 Tongue Squamous cell carcinoma	Sonovue/0.3 ml Peritumoral	Surgical pathology	The identification rate of the sentinel nodes was 91.7%. 15 SLN were found in 11 cases (1.4 SLN/patient).
Lahtinen et al. 2018 Finland	*N* = 12 Vulva Squamous cell carcinoma	Sonovue/0.4 ml Upper lateral of mons pubis	Surgical pathology	To compare to routing SLN biopsy, overall sensitivity was 81.2% (13/16). CEUS detected enhancing SLN in 2 cases not seen by traditional method. All metastatic SLNs (*n* = 5) were correctly identified by CEUS procedure.

a sensitivity of 94.7%, a specificity of 70.0% and an accuracy of 89.6% for the use of lymphosonography to characterize metastatic SLNs.

Outside of the head and neck region there was only one other clinical study. Lahtinen et al. [66] conducted a pilot study in 12 patients with vulvar SCC. The UCA Sonovue was injected into the upper lateral side of the mons pubis with the patients undergoing preoperatively inguinal CEUS SLN examination and guide wire marking of the enhanced SLNs. During surgery, the guide-wire marked CEUS-positive SLNs were identified and compared to the radioactive tracer and/or blue dye findings. The results showed a sensitivity of 81.2% (13/16) for CEUS SLN identification when compared with radioactive tracer and/or blue dye SLNs findings. All metastatic SLNs (5 out of 5 in total) were correctly identified by lymphosonography.

Conclusion

This paper reviewed and summarized the literature on the clinical use of contrast lymphosonography for SLN identification for cancer localization and staging. The overall majority of clinical trials on the subject was performed in patients with breast cancer. The results from use of lymphosonography in the studies described in this review showed the great potential for this technique to become part of the clinical standard of care.

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Conflict of Interest

The authors have no conflict of interest related to this review article to report.

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