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Contrast-enhanced Ultrasound Using Intradermal Microbubble Sulfur Hexafluoride for Identification of Sentinel Lymph Nodes During Breast Cancer Surgery: A Clinical Trial

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Abstract. *Background/Aim: Sentinel lymph node (SLN) procedures have gained popularity in early breast cancer thanks to the reduction of surgical side-effects. The standard SLN mapping procedure uses ^{99m}Tc-nanocolloid human serum albumin with/without blue dye; limitations include logistical challenges and adverse reactions. Recently, contrast-enhanced ultrasound (CEUS) using sulfur hexafluoride has emerged as a promising technique for SLN mapping. Our study aimed to compare the CEUS technique with the standard isotope method. Materials and Methods: AX-CES, a prospective, monocentric, single-arm phase-3 study was designed (EudraCT: 2020-000393-20). Inclusion criteria were histologically diagnosed early breast cancer eligible for upfront surgery and SLN resection, bodyweight 40-85 kg, and no prior history of ipsilateral surgery or radiotherapy. All patients underwent CEUS prior to surgery and blue dye injection was performed in areas with contrast accumulation. After the experimental procedure, all patients underwent the standard mapping procedure and SLN frozen section assessment was performed. Data on the success rate, systemic reactions, mean procedure time, CEUS appearance, SLN number, and concordance with standard mapping procedure were collected. Results: Among 16 cases, a median of two SLNs were identified during CEUS. In all cases, at least one SLN was identified by CEUS (100%). In six cases, SLNs were classified during CEUS as abnormal, which was confirmed by definitive staining in four cases. After the standard mapping technique, in 15 out of the 16 cases (87.50%), at least one SLN from the standard mapping procedure was marked with blue dye in the CEUS procedure. In our series, sensitivity and specificity of SLN detection by CEUS were 75% and 100%, respectively. Conclusion: CEUS is a safe and manageable intraoperative procedure. When compared with standard techniques, US appearance during CEUS may provide additional information when associated with histological assessment.*

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Key Words: Sentinel lymph node, sentinel lymph node biopsy, breast neoplasm, ultrasound, mammary, ultrasonography, contrast-enhanced ultrasound, axillary imaging, lymphoscintigraphy.

Breast cancer (BC) represents the leading oncological diagnosis among the female population (1). Despite the global burden, in the past 30 years, a multidisciplinary approach has led to a steady reduction in mortality (2, 3). Additionally, BC screening programs have allowed a higher rate of early-stage diagnosis, enabling de-escalation in medical and surgical treatment (4, 5). However, despite a deeper knowledge of etiopathogenesis and development of biological markers for BC, axillary involvement is still the leading prognostic factor affecting clinical decisions after surgery (6-9).

Among axillary treatments, sentinel lymph node (SLN) procedures, introduced by Krag *et al*. (10) and Giuliano *et al*. (11) in 1993 and 1994, respectively, gained popularity in early BC (EBC) thanks to its reduction of side-effects (such as ipsilateral upper arm lymphedema, restriction of movement, and arm and chest wall numbness) (12).

The SLN is defined as the first draining LN station from the breast lesion. ^{99m}Tc-Nanocolloid human serum albumin and blue dye staining are the most common mapping methods (13). With a detection rate of 96% when both methods were combined (14), isotope limitations include logistical challenge for the facilities such as handling and disposal of radioactive drugs, short half-life, training of staff, and legislative requirements (13, 15). In contrast, use of the most popular alternative, blue dye, is associated with a higher false-negative rate and other limitations, including systemic or local adverse reactions, and genotoxic *in vitro* effects (13, 16, 17).

To overcome these limitations, some authors began to explore new SLN tracers, applying different technologies as indocyanine green, or contrast-enhanced ultrasound (CEUS). Regarding CEUS, this technique is routinely used in hepatic US, enhancing conventional US procedures (18). In order to evaluate this promising technique, in our study we aimed to compare the standard mapping procedure, namely ^{99m}Tc-nanocolloid human serum albumin, with the CEUS procedure using intradermal sulfur hexafluoride microbubbles.

Materials and Methods

Study design and patient selection. A prospective, monocentric, interventional phase-3 study, single-arm, non-inferiority clinical trial named AXillary Contrast-Enhancement ultraSound evaluation (AX-CES) was designed (Figure 1). Primary endpoints of the study were defined as the evaluation of the technical applicability of CEUS in clinical practice and its concordance rate with the standard mapping procedure. The local Institutional Review Board of Tor Vergata approved the study (AX-CES 1.2020) and the clinical trial was registered (EudraCT code: 2020-000393-20). AX-CES 1.2020 was funded by Italian Ministry of Health (CUP N:E84119002750006). The initial study was designed as a pilot study and enrollment was set at 25 patients.

Primary inclusion criteria for the AX-CES study were histologically proven EBC, age >18 years, and bodyweight between 40 and 85 kg. EBC was defined as cT0-2 cN0 cM0 in patients according to the ACOSOG Z0011 criteria (19-21), and only patients eligible for breast-conserving surgery and upfront SLN dissection were included in the study. Conversely, patients with prior radiation therapy or surgery for any reason in the ipsilateral breast or axilla were excluded from the study. Moreover, patients under medical treatment which might impair endocrine status or the immunological system were also excluded from the study (22, 23). Pregnancy, hematoma or inflammatory disease of the ipsilateral breast, axilla, or upper arm, as well as a personal history of hypersensitivity to any drug involved in the protocol were considered other exclusion criteria. According to these prerequisites and requirements, the AX-CES study lasted from October 2020 to December 2021, once the patients' enrollment was completed.

Preoperative assessment. After written consent was obtained, preoperative assessment of the patient was performed, and demographic and preoperative data were collected. Due to the design of the study, all patients were informed that no randomization was needed, and that the experimental procedure (CEUS) would be performed at the same time as the standard mapping procedure. Prior to the CEUS procedure, all patients underwent lymphoscintigraphy up to 12 h before surgical procedure with subdermal injection of the radioactive isotope ^{99m}Tc-nanocolloid human serum albumin (Nanocoll; GE Healthcare, Chicago, IL, USA) with 40 mBq in the periareolar, upper outer quadrant region. Numbers of LNs with radioisotope accumulation were collected and blinded to surgeons and radiologists involved in the study protocol.

Experimental procedure: CEUS. CEUS was performed according to modified Sever techniques (24). After admission to the Surgical Department and continuous monitoring of vital signs, a radiologist with more than 10 years' experience in breast pathology performed axillary US examination with high-resolution US equipment (MyLab Twice; Esaote, Genoa, Italy), and a high frequency linear-array probe (10-13 MHz) operating at 7 MHz with a mechanical index of 0.30. Neither the breast radiologist nor patients were aware of the results of lymphoscintigraphy (double-blind study).

Conventional grey-scale axilla US was performed to locate LNs before US contrast administration. Subsequently, after injection of peripheral anesthetic (2% lidocaine, 5 ml), 0.2-0.4 ml of sulfur hexafluoride (Sonovue®; Bracco Imaging, Milan, Italy) (Figure 2) were injected into the upper outer periareolar area (Figure 3). Identification of SLNs with CEUS was performed with Cadence Pulse Sequencing software package (MyLab Twice; Esaote), and dual images (gray-scale tissue and contrast agent image) were obtained to confirm an architecturally defined LN in areas of sulfur hexafluoride accumulation (Figure 4). The morphological appearance of LNs in the area of sulfur hexafluoride accumulation was registered as normal or pathological. After CEUS, blue dye was injected into the area of sulfur hexafluoride accumulation. If different areas of sulfur hexafluoride accumulation were recognized during CEUS, blue dye injection was performed in all areas.

Periprocedural data collection included adverse drug reactions, and number of LNs visualized. After the procedure, the breast radiologist had to leave the Surgical Department from a different exit to avoid any contact with the breast surgeon (double-blind).

Surgical procedure and postoperative care. After CEUS, all patients underwent breast-conserving surgery by a breast surgeon with more than 10 years of experience in breast surgery. The surgeon was unaware of the CEUS result (double-blind). All patients were placed in *supine decubitus* with the arm open at 90˚ on the side of the operation. Two different incisions were performed for quadrantectomy and SLN biopsy. Breast-conserving surgery was performed in the standard fashion depending on the breast volume and BC volume with wire-guided localization if needed preoperatively (25, 26). Frozen sections were performed in cases of clinical suspicion. If patients underwent mastectomy due to margin involvement, they were excluded from the study. A second incision was performed on the anterior axillary line. A neoprobe gamma detection system (Mammotome, Cincinnati, OH, USA) was used to identify the hottest node. After *ex vivo* LN evaluation, other LNs were removed following the '10% rule' (all LNs with counts >10% of *ex vivo* count of the most radioactive node should be removed) (27), or if abnormal. The number of LNs was determined with the standard mapping procedure. After standard mapping, the number of LNs harvested with isotope which were marked during CEUS were analyzed separately.

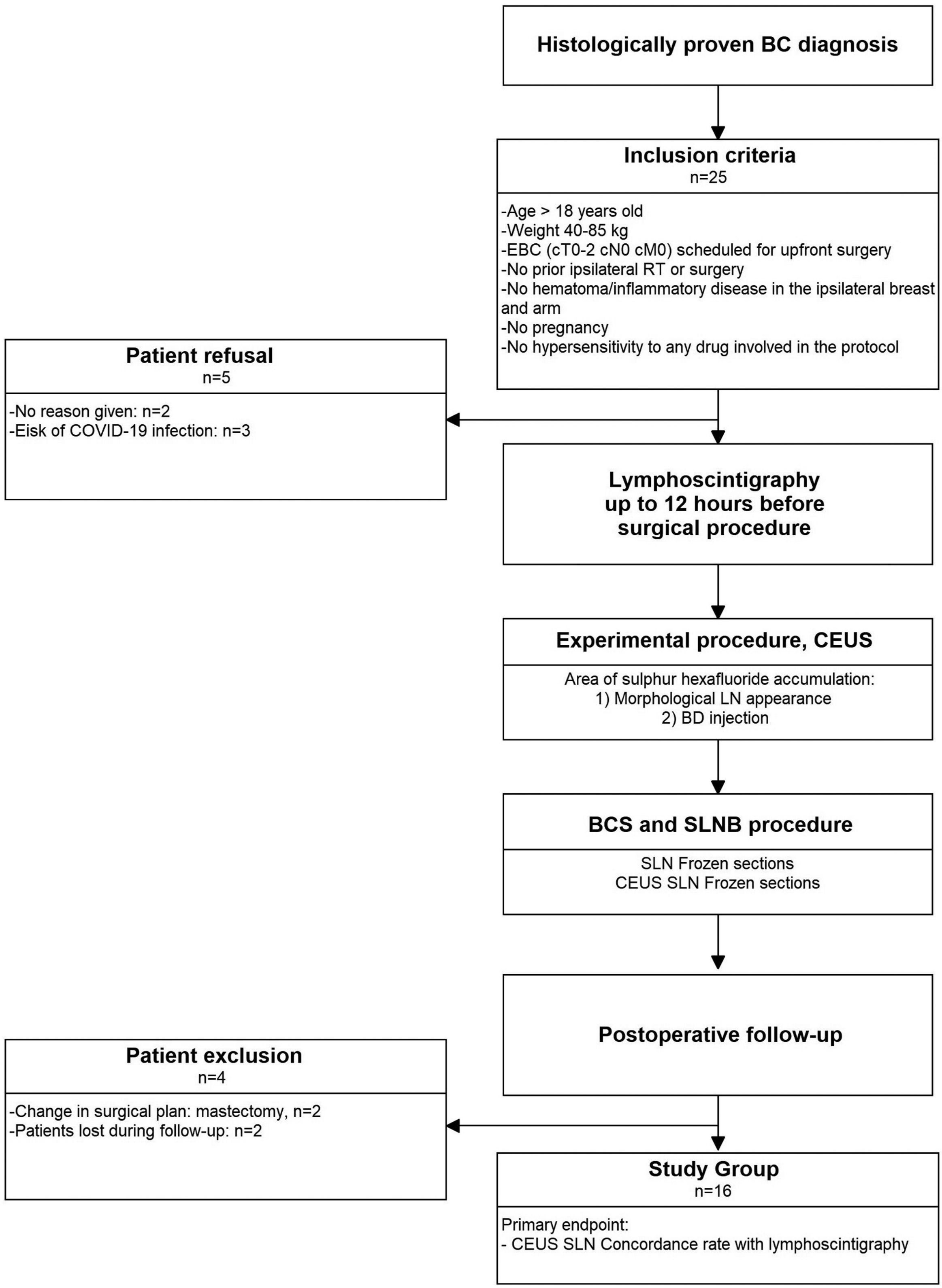

Figure 1. *AX-CES 1.2020 study flowchart. BC: Breast cancer; BCS: breast-conserving surgery; BD: blue dye; CEUS: contrast-enhanced ultrasound; COVID-19: coronavirus disease 19; EBC: early breast cancer; LN: lymph node; RT: radiotherapy; SLN: sentinel lymph node; SLNB sentinel lymph node biopsy.*

Frozen section of all SLNs was performed and when a metastasis was present in more than two LNs, axillary LN dissection (ALND) was carried out. During the surgical procedure, fluid infusion at 1.5 ml/kg/h of normal saline and Ringer's solution were used. After surgery, fluid infusion was stopped within 2 hours and oral intake was allowed immediately if tolerated.

Data collection and postoperative follow-up. After surgery, all patients were evaluated on the first, second, and thirtieth postoperative days. All surgical complications were recorded according to the Clavien–Dindo classification (28). Patient data from pathological examinations were analyzed regarding the type of neoplasia, tumor dimensions, surgical margins (in millimeters), number of LNs evaluated and their evaluation. Nodal involvement was defined as negative (free from disease), micrometastatic (metastasis ≤2 mm or isolated tumor cells) or macrometastatic (metastasis >2 mm). Estrogen receptor, progesterone receptor and Ki67 protein were expressed as a percentage, overexpression of human epidermal growth factor receptor 2 expression (HER2) was classified according to the 2018 recommendations by the American Society of Clinical Oncology/College of American Pathologists Clinical Practice (29).

Statistical analysis. All data were recorded onto an EXCEL database (Microsoft, Redmond, WA, USA). Continuous variables were reported as median and interquartile range, Dummy variables reported as numbers and percentages. SPSS statistical package version 23.0 was used (IBM Corp., Armonk, NY, USA). US appearance, standard mapping, and blue dye-marked standard-mapping frozen section SLN were compared with definitive staining of the standard mapping procedure in terms of sensitivity, specificity, accuracy, positive predictive value, negative predictive value, and false-negative rate. Continuous variables between procedures were compared with Mann–Whitney *U*-test, while for categorical variables Fisher exact test was applied.

Results

Baseline data. A total of 25 patients were considered for being enrolled, but five refused: three patients due to the risk of COVID-19 infection, and in two cases, no reason was given. The enrollment of patients since January 2020 strongly decreased because of the COVID-19 pandemic, which led to a reduction in the acceptance rate of patients (30-33). The COVID-19 pandemic strongly affected daily clinical practice in several medical specialties, such as transplant surgery (34, 35). After enrollment, four patients were excluded from the study: Two cases were excluded due to the intraoperative surgical plan (mastectomy), and two other patients were excluded postoperatively because they did not complete postoperative follow-up due to COVID-19 infection.

Hence, 16 patients were included in the study. Patient characteristics included in the analysis are enlisted in Table I. Baseline variables demonstrated a median age of 57.75 (40.50-75.75) years. Mean operative time without CEUS was 123.24 (114.50-134.50) min. Postoperative assessment confirmed the preoperative diagnosis of BC. 12 cases were classified as invasive ductal BC, two as lobular BC, and two as other types (one case of tubular BC and one case of medullary BC). Regarding localization, 12 cases were diagnosed in the outer quadrants and four in the inner quadrants. Among these cases, eight were defined as multicentric/multifocal BC. Patients were classified according to TNM as T1 in 10 and T2 in the remaining six. No case of ductal carcinoma in situ was documented, but in five (31.25%) patients, BC surrounded by ductal carcinoma in situ was described.

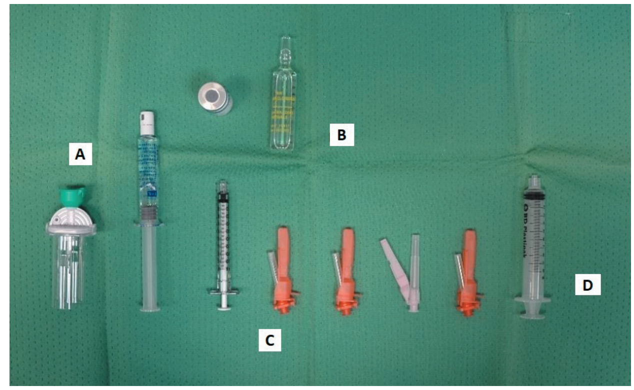

Figure 2. *Contrast-enhanced ultrasound materials. A: SonoVue® package. B: 2% Lidocaine (20 mg/ml, 10 ml) which were used for local anesthesia. C: 26-G Needles and a 1-ml tuberculin syringe used for microbubble suspension. D: Needles (26-G and 21-G) and a 10-ml syringe for local anesthesia injection.*

Regarding postoperative follow-up, a total of seven complications were recorded: Four were classified as Clavien–Dindo grade <2 (two cases of seroma treated conservatively, one case of postoperative pain, and one case of postoperative anemia treated conservatively), and three as Clavien–Dindo grade ≥2 (two cases of postoperative seroma which required needle aspiration, and one case of abscess which required medical treatment and needle drainage).

CEUS. Among 16 cases, a total of 29 LNs were identified pre-operatively during CEUS and marked with blue dye. In all 16 cases, at least one SLN was identified by CEUS, and blue dye injection was performed. The median number of LNs identified was 2. The mean duration of the procedure was 18.87 min. Interestingly, the mean procedure time recorded for the first five patients was statistically significantly longer than for the last five patients enrolled (23.46 *vs*. 17.26 min; *p*=0.011). In six patients, suspicious LNs were identified, which they were not detectable with prior CEUS procedure. After the CEUS procedure, neither side-effects nor complications were reported.

SLN procedures. After CEUS, the standard mapping procedure was carried out. The isotope technique detected a

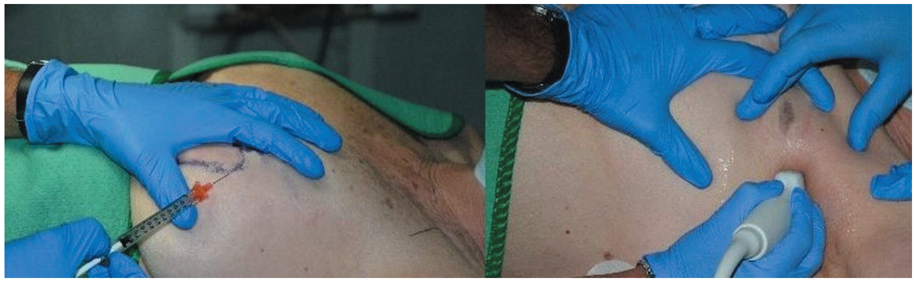

Figure 3. *Contrast-enhanced ultrasound procedure. From left to right. Contrast agent injection and axillary ultrasound. On the left breast, the preoperative design (left batwing quadrantectomy) is displayed.*

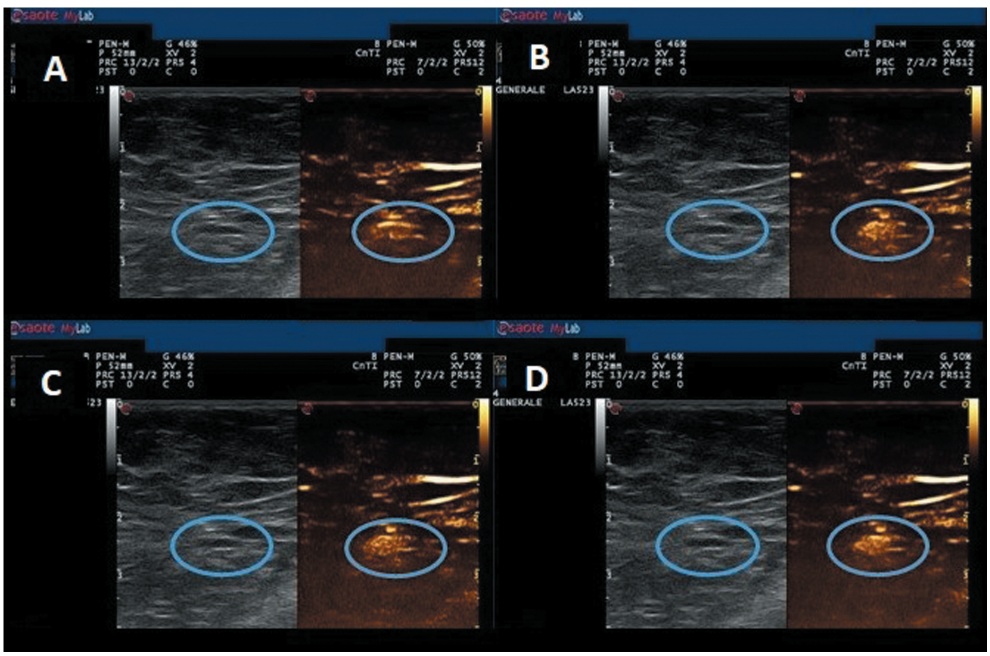

Figure 4. *Axillary node enhanced in contrast-enhanced ultrasound (blue circle) at 15 (A), 25 (B), 35 (C) and 45 (D) seconds after 0.2 ml injection of Intradermal microbubble sulfur hexafluoride in the periareolar space.*

total of 40 LNs. The median number of SLNs detected by standard mapping was statistically significantly higher than that detected by CEUS (2.5 *vs*. 2, respectively; $p=0.025$). Moreover, in 15 out of 16 cases, at least one SLN harvested in the standard mapping procedure (hottest node/abnormal LN/10% rule LN) was marked with blue dye in the CEUS procedure. The total number of SLNs detected by CEUS was 26 and a subanalysis of these revealed five patients had at least one positive SLN by CEUS.

Table II summarizes the results of the different procedures when compared with the gold standard technique (definitive assessment of the SLN specimen). Table III shows the sensitivity, specificity, accuracy, positive predictive value, negative predictive value, and false-negative rate of CEUS, frozen sections of CEUS-detected SLNs, and frozen sections of SLNs by standard mapping. In this analysis, micrometastatic LNs and those with isolated tumor cells were considered negative for this analysis.

CEUS demonstrated a lower sensitivity (66.7%) and specificity (80%) when compared to standard mapping procedure. Interestingly, CEUS failed to identify two positive cases which were classified as negative during CEUS. The first case resulted in a single macrometastatic LN and in the second case, two positive LNs were classified as macrometastatic out of four LNs collected. In both cases, due to the lower disease burden, ALND was not performed.

Interestingly, sensitivity (75.00%) and specificity (100%) for CEUS-detected SLNs was higher. In a single case, assessment

Table I. *Baseline preoperative and intraoperative variables.*

Variable	Study group (n=16)
Mean age (IQR), years	57.75 (40.50-75.75)
Mean BMI (IQR), kg/m^2	23.51 (21.28-25.89)
Mean hospital stay (IQR), days	1.93 (1.52-2.57)
Mean operative time (IQR), min	123.24 (114.50-134.50)
Clavien–Dindo complication rate, n (%)	
Grade <2	4 (25.00%)
Grade ≥2	3 (18.75%)
Tumor diameter, cm	1.95 (1.51-2.64)
Tumor location, n (%)	
Outer quadrants	12 (75.00%)
Inner quadrants	4 (25.00%)
Tumor distribution, n (%)	
Unifocal BC	8 (50.00%)
Multicentric BC	5 (31.25%)
Multifocal BC	3 (18.75%)
Histological type, n (%)	
Ductal	12 (75.00%)
Lobular	2 (12.50%)
Other	2 (12.50%)
Receptor status	
Mean ER (IQR)	48.06% (16.00-82.50)
Mean PR (IQR)	51.19% (35.00-73.00)
Proliferating factor, Ki67	28.94% (20.75-36.50)
Tumor grade, n (%)	
I	8 (50.00%)
II	6 (37.50%)
III	2 (12.50%)
HER 2 score, n (%)	
Grade I	8 (50.00%)
Grade II	7 (43.80%)
Grade III	1 (6.30%)
DCIS, n (%)	
Yes	5 (31.25%)
No	11 (68.80%)
Definitive SLN assessment (n=16), n (%)	
Macrometastatic	6 (37.50%)
Micrometastatic	2 (12.50%)
ITC	1 (6.30%)
Negative	7 (43.80%)
LN from standard mapping painted with BD (n=16), n (%)	
Yes	15 (87.50%)
No	1 (12.50%)

BMI: Body mass index; DCIS: ductal carcinoma *in situ*; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; IQR: interquartile range; ITC: isolated tumor cells; LN lymph node; PR: progesterone receptor; SLN sentinel lymph node.

of CEUS-detected SLNs failed to predict ALN status. Interestingly this patient was negative during standard mapping and no ALND was performed in the first procedure. Additionally, as mentioned above, in one patient, no CEUS-detected SLNs were collected during standard mapping. Due to this case, standard mapping demonstrated a higher sensitivity (83.3%) when compared with CEUS detection of SLNs.

Discussion

SLN evaluation represented a breakthrough in BC surgery, providing equivalent oncological safety in EBC when compared with ALND with reduction of the surgical extent (10, 11, 32, 33). However, despite the lower complication rate, SLN assessment is associated with surgical complications similar to those of ALND due to the dissection of axillary lymphatic channels, such as arm lymphedema (5%) and sensory loss (18% at 1 month) (12). Another limitation of SLN biopsy is the lack of stratification among patients. After surgery, definitive SLN biopsy will eventually be negative in up to 70% of patients with EBC (15, 36-38), suggesting that SLN biopsy might be avoided in a larger number of patients without detrimental effects. In the present study, CEUS was found to be a potentially innovative technique for axillary staging.

After the practice-changing ACOSOG Z0011 trial (21), which suggested that there was no significant benefit of ALND in patients with EBC with up to two positive SLNs, the rates of ALND and intraoperative evaluation of SLNs significantly declined. In light of this, the SOUND trial aimed to evaluate oncological outcomes of SLN biopsy avoidance in patients with EBC (39) and a recent sub-analysis from this study demonstrated the detrimental effect of axillary BC on the physical function of the ipsilateral upper limb (40).

To reduce complications and side-effects as much as possible, identification of alternative techniques for stratifying patients is urgently needed to reduce unnecessary axillary surgery. Under these circumstances, CEUS may be useful prior to surgery to identify patients who will need SLN biopsy or could safely avoid it. The present study aimed to investigate the role of CEUS as a novel tracer of SLNs and its possible role preoperatively.

Conventional US is a routine imaging modality combined with mammography prior to surgery for assessing breast lesions and ALN status (41). Dimensional, morphological, and color Doppler characteristics are routinely used in clinical practice to predict ALN status but with low specificity and sensitivity (42). However, as mentioned before, LN disease will be underestimated in up to 30% patients with conventional US, with detrimental effects on clinical care and clinical decision-making. It is safe to assume that even if a large proportion of these patients meet the Z0011 criteria (21, 43), a significative proportion with positive LNs may be misclassified as having EBC, missing the chance for neoadjuvant medical therapy prior to surgery (44, 45).

When compared with morphological US, CEUS provided additional information when contrast agent was injected into a vein (46). Many authors underline how CEUS features can be combined with conventional imaging to characterize the ALN status (24, 46), in particular, different enhancement

Table II. *Metastatic status of sentinel lymph nodes (SLNs) detected by contrast-enhanced ultrasound (CEUS) appearance, by frozen section from standard mapping (injection of radioactive isotope, ^{99m}Tc-nanocolloid human serum albumin), and in the lymph node enhanced by CEUS and standard mapping.*

			ALN metastasis, n	
CEUS	Suspicious appearance	Yes	4	2
		No	2	8
Standard mapping	Macrometastasis in frozen section	Yes	5	0
		No	1	10
CEUS SLN	Macrometastasis in frozen section	Yes	4	0
		No	1	10

ALN: Axillary lymph node.

Table III. *Diagnostic efficiency of contrast-enhanced ultrasound (CEUS) appearance of sentinel lymph nodes (SLNs) and, frozen section from standard mapping (injection of radioactive isotope ^{99m}Tc-nanocolloid human serum albumin).*

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CEUS		66.70%	80.00%	66.70%	80.00%
Frozen section	Standard mapping	83.30%	100.00%	100.00%	90.91%
	CEUS SLN	75.00%	100.00%	100.00%	90.91%

NPV: Negative predictive value; PPV: positive predictive value.

patterns were recognized by several authors after periareolar injection (24, 47). In our analysis, although the enhancement pattern was not evaluated, in six patients a LN was defined as suspicious after CEUS while conventional US classified it as negative, providing potential additional preoperative or intraoperative information. Additionally, a potential benefit of US guided procedure is its potential integration with percutaneous procedures, such as core-needle biopsy or fine-needle aspiration.

Intraoperative US for BC, since its first description in 1988 (48), soon became an accurate and effective tool for localizing breast masses visualized by US. It resulted in lower rate of negative margins, reducing the re-excision rate, reducing patient complications, and providing better esthetic outcomes (49-51). Moreover, in recent years, novel US-guided interfascial plane blocks were introduced into clinical practice by anesthesiologists and surgeons to obtain better postoperative pain control (52-54). Due to the popularity of these procedures, breast US soon became a useful instrument in the clinical practice of breast surgeons. Among this application, CEUS may represent another implementation of US in the surgical armamentarium with a short learning curve. In our series, the mean duration of CEUS was 18.28 min, comparable to another study (55), and the procedure was successfully performed in our series. Thus, in our series, CEUS resulted in a cost-saving, manageable procedure. Moreover, no skin reactions around the periareolar injection site, nor systemic allergic reaction were observed, demonstrating that sulfur hexafluoride, even though designed as an intravenous agent, is safe when injected intradermally.

As expected from previous evidence (56), a statistically significantly lower number of SLNs were reported during CEUS when compared with standard mapping procedures. However, these findings need to be interpreted carefully. First of all, as is largely known, US is highly operator-dependent (57), and some of these results may have been influenced by the number of procedures performed by our radiologists. It has been calculated that experience of 25 cases is needed for a physician to master this technique (55), thus we expect better performance with increasing experience.

Although a lower median number of SLNs was reported with CEUS, the number of SLNs to be harvested during breast surgery is not formally standardized. Since the introduction of SLN biopsy, there has been controversy regarding the minimum number of SLNs needed to be able to predict the ALN status (58). Several factors have been linked to the number of SLNs harvested, such as age, preoperative chemotherapy, and surgeon (59). A recent multivariate analysis by Dixon *et al*. demonstrated the pivotal role of the surgeon, describing a highly variable number of LNs harvested during SLN evaluation (59). As mentioned before, the SLN is defined as the first ALN that receives lymphatic flow from the primary tumor. While theoretically just one LN should be defined strictly as the SLN, it is widely accepted that this includes some of the LNs receiving lymphatic drainage from the first SLN (60, 61). In fact, despite a higher false-negative rate, the removal of a lower number of SLNs is not associated with poor local disease control and axillary recurrence (62, 63).

Regarding the concordance of CEUS results with those of standard mapping, in our series, in 15/16 cases, at least one SLN found in standard mapping was marked during CEUS, showing its potential validity in clinical practice. Although this may raise some concerns regarding the procedure, it is important to underline some aspects. First of all, the false-negative rate after standard mapping of SLNs is 5-10% (62, 64). A higher SLN false-negative rate has been associated with multicentric, larger tumors, and after primary medical treatment (65). Moreover, in a recent monocentric study, the false-negative rate of frozen section assessment when compared with the final paraffin-section report was 12.6% (66). Compared with these, we believe that the CEUS technique represents a valuable strategy, thanks to the integration between the appearance of LNs in intraoperative US and histological assessment.

Additionally, even if a higher risk of residual disease may be present, although in one case CEUS detection of SLNs failed to predict macrometastases, no ALND was performed due to the lower disease burden, and the patient underwent only SLN biopsy. As mentioned before, the ACOSOG Z0011 trial demonstrated how EBC in patients with a low disease burden in the axilla may be controlled with adjuvant treatments and nutritional support (21, 67). Further studies are needed to assess and confirm these preliminary results, which could determine the role of CEUS in the outpatient clinic, as a non-invasive preoperative procedure, or in the surgical theatre as unique SLN-mapping technique. Moreover, further studies will address its role in addition to other conventional standard mapping techniques in specific clinical settings such as surgery after primary medical therapy.

We are aware that some limitations may have influenced our results. To begin, the small sample size may have affected our findings. However, our research was designed as a pilot study to investigate the clinical application and concordance rate of CEUS with standard mapping and no power analysis was performed. A larger study will be designed to confirm our preliminary data in the outpatient clinic. Another possible source of error was linked with the oncological features of the patients enrolled in the study. Due to the inclusion criteria, most of the patients enrolled may have been classified at being at low risk of axillary involvement. However, strict clinical inclusion criteria were designed to enroll patients with EBC with a clear surgical indication for upfront surgery. Additional studies with larger series may investigate the role of CEUS in specific populations, such as patients treated with neoadjuvant chemotherapy or patients with a higher risk of axillary involvement.

In conclusion, despite these limitations, in our work we demonstrated how the CEUS technique is a safe and manageable intraoperative procedure. When compared with standard techniques, US appearance after CEUS may provide additional information when associated with histological assessment. However, larger series of multicentric cohorts are required to obtain long-term outcomes and to determine the safety and the therapeutic effect of alternatives to SLN biopsy, such as outpatient CEUS-guided SLN core-needle biopsy or fine-needle aspiration.

Conflicts of Interest

The Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

Conceptualization: Oreste Claudio Buonomo, Gianluca Vanni, Marco Materazzo. Data curation: Marco Pellicciaro, Giada Iafrate, Benedetto Longo and Benedetto Ielpo. Formal analysis: Stefano Rizza, Chiara Adriana Pistolese and Perretta Tommaso. Investigation: Oreste Claudio Buonomo, Marco Materazzo, Rosaria Meucci, Benedetto Longo and Valerio Cervelli. Project administration and Supervision: Gianluca Vanni. Writing - original draft: Marco Materazzo and Oreste Claudio Buonomo. Writing - review and editing: Gianluca Vanni and Giada Iafrate. All Authors read and agreed to the submitted version of the article.

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