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Contrast-enhanced Ultrasound Using Intradermal Microbubble Sulfur Hexafluoride in Non-invasive Axillary Staging in Breast Cancer: Are we Missing a Chance?

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Abstract. *Background/Aim: In the context of surgical de-escalation in early breast cancer (EBC), this study aimed to evaluate the contrast enhancement ultrasound (CEUS) sentinel lymph node (SLN) procedure as a non-invasive axillary staging procedure in EBC in comparison with standard SLN biopsy (SLNB). Patients and Methods: A subanalysis of the AX-CES study, a prospective single-arm, monocentric phase 3 study was performed (EudraCT: 2020-000393-20). The study included patients with EBC undergoing upfront surgery and SLN resection, with no prior history of locoregional treatment, and weighing between 40-85 kg. All patients underwent the CEUS SLN procedure as a non-invasive axillary staging procedure, with CEUS SLN accumulation marked using blue dye. After the CEUS SLN procedure, all patients underwent the standard mapping procedure. Data on success rate, systemic reactions, mean procedure time, mean surgical procedure, mean procedure without axillary staging, CEUS SLN appearance (normal/pathological), SLN number, and concordance with standard mapping procedure were collected. Results: After the CEUS SLN procedure, 29 LNs among 16 patients were identified and marked. In all cases, CEUS SLN revealed at least one LN enhancement. Six (37.50%) LNs were defined as pathological after the CEUS SLN procedure. Definitive staining of CEUS SLN pathology revealed metastatic involvement in four (66.67%) of the cases. Two SLNs were identified during the CEUS SLN procedure; however, owing to the low disease burden, no change in the surgical plan was reported. Conclusion: The CEUS SLN procedure shows promise as a technique for non-invasive assessment of the axilla, potentially enabling safe axillary de-escalation in EBC by estimating the axillary disease burden.*

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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) is the leading oncological diagnosis in the female population (1). Among prognostic factors, axillary status has been classically associated with poor locoregional and distant disease control (2). In this context, axillary surgical exploration has traditionally achieved two main goals: locoregional staging and disease control (3). However, in the last 20 years, a multidisciplinary approach guaranteed a safe de-escalation in axillary surgery, aiming to reduce surgical sequelae (4).

Sentinel lymph node (SLN) biopsy, described by Krag and Veronesi, represented the first attempt to reduce surgical extent in the axilla in early BC (EBC) (5, 6). In addition, the results of the ten-year ACOSOG Z0011 trial demonstrated how SLN biopsy (SLNB) alone, in the context of multidisciplinary treatment, could contribute to controlling limited axillary disease (7). These results determined a progressively diminished therapeutic role of axillary surgical exploration, emphasizing to a more staging-oriented role in EBC.

Moreover, in recent years, genomic tests for BC have assertively entered in the clinical practice to assess relapse risk through intrinsic biological behavior in addition to the other well-known risk factors. Given this innovation, some authors argued that surgical axillary exploration may not be always mandatory for treatment decision-making process and disease control (8). The SOUND study demonstrated how an axillary ultrasound in selected patients could not have a detrimental effect on treatment decision-making process and disease control, reducing surgical sequelae and side effects (9, 10).

However, axillary ultrasound (US) has a drawback due to the wide range of sensitivity (24%-94%) attributed to operator dependency and technical difficulty in accessing the axillary tissue (11-13). Therefore, to enhance US performance, several technologies have been implemented in the clinical practice, such as sonoelastography or doppler, and are currently widely applicated (14, 15). Recently, among different US modalities, contrast enhancement US (CEUS) has emerged as an innovative technique to detect SLN and perform SLN biopsy. Additionally, CEUS SLN, when compared with other SLN tracers, may provide additional preoperative and intraoperative information (16). Therefore, in the context of surgical de-escalation from the SOUND study, the aim of the present study was to evaluate the role of the CEUS SLN procedure as non-invasive axillary staging procedure in EBC in comparison with standard staging axillary procedure (SLN).

Patients and Methods

Study design and patient selection. A subanalysis from a prospective, monocentric, interventional phase 3-study, single arm, non-inferiority clinical trial named AXillary Contrast-Enhancement ultraSound evaluation (AX-CES) was planned (EudraCT code: 2020-000393-20). The Local Institutional Review Board of Tor Vergata approved the study (AX-CES 1.2020), which was funded by Italian Ministry of Health (CUP N: E84119002750006). The primary endpoints of the study were technical applicability of CEUS and concordance rate with standard technique; the present study aimed to evaluate CEUS SLN procedure predictability of axillary lymph node (LN) involvement. The AX-CES 1.2020 subanalysis flowchart is displayed in Figure 1, and enrollment was set at 25 patients.

In AX-CES 1.2020, patients with age >18 and histologically proven EBC defined as cT0-2cN0cM0, eligible for upfront breast conserving surgery (BCS) and SLNB as defined in the Z0011 study were included (7). Patients who received prior thoracic radiation therapy, ipsilateral breast or axillary surgery, were excluded, as well as patients with a personal history of hypersensitivity to any drug involved in the protocol. Following these conditions, the AX-CES Study lasted from October 2020 to December 2021, once the patient enrollment was completed.

Preoperative assessment and surgical procedure. After written consent, prior to the experimental procedure (CEUS SLN), all patients underwent lymphoscintigraphy up to 12 hours before the surgical procedure with the 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. The number of LNs with radioisotope accumulation was collected and blinded to surgeons and radiologists involved in the study protocol. If the patient's surgical procedure was scheduled in the morning, hospital admission occurred the day before surgery.

Experimental procedure: CEUS SLN. A Radiologist with more than 10 years of experience in Breast Imaging performed Axillary US 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. Prior CEUS, conventional 2D US was performed to locate LNs in the surgical theatre before surgery.

As in the CEUS SLN procedure described by Sever *et al.* (17), anesthesia was obtained by injecting with a 25-G needle 3 ml of 2% Lidocaine, 0.2-0.4 ml of reconstituted sulfur hexafluoride (Sonovue®; Bracco Imaging, Milan, Italy) solution and 3 ml of saline (0.9% NaCl into the subcutaneous layer of the areola in the upper outer quadrant. The identification of CEUS SLNs was made using Cadence Pulse Sequencing software package (MyLab Twice; Esaote, Genoa, Italy). Dual Image was obtained to confirm an architecturally defined LN in the contrast accumulation area to assess CEUS SLN procedure morphology. The morphology obtained using the CEUS SLN procedure was registered as normal or pathological. The morphology identified using the CEUS SLN procedure was defined pathological when one of the following conditions were satisfied: cortical thickness >3 mm, eccentric thickening, irregular margin, eccentric displacement of fatty hilum, small vessels entering cortex of node (color flow ultrasound), enlarged node with no fatty hilum, or rounded shape. At the end of the CEUS SLN procedure, blue dye was injected into all area of CEUS accumulation.

Intraoperative and postoperative management. Intraoperative management was performed in supine decubitus position with the arm abducted to 90˚ degree on the side of surgery. Two surgical incisions were performed for BCS and SLN. BCS were performed according to the BC Breast Volume ratio and margin involvement. Margin frozen section assessment was performed in case of clinical suspicion. If patients underwent mastectomy, they were excluded from the study. During axillary surgery, SLNB was carried out according to the hottest LN identified with the gamma-probe detection system (Neoprobe detection system, Danaher Corporation, Washington, DC, USA). After *ex vivo* SLN evaluation, other LNs

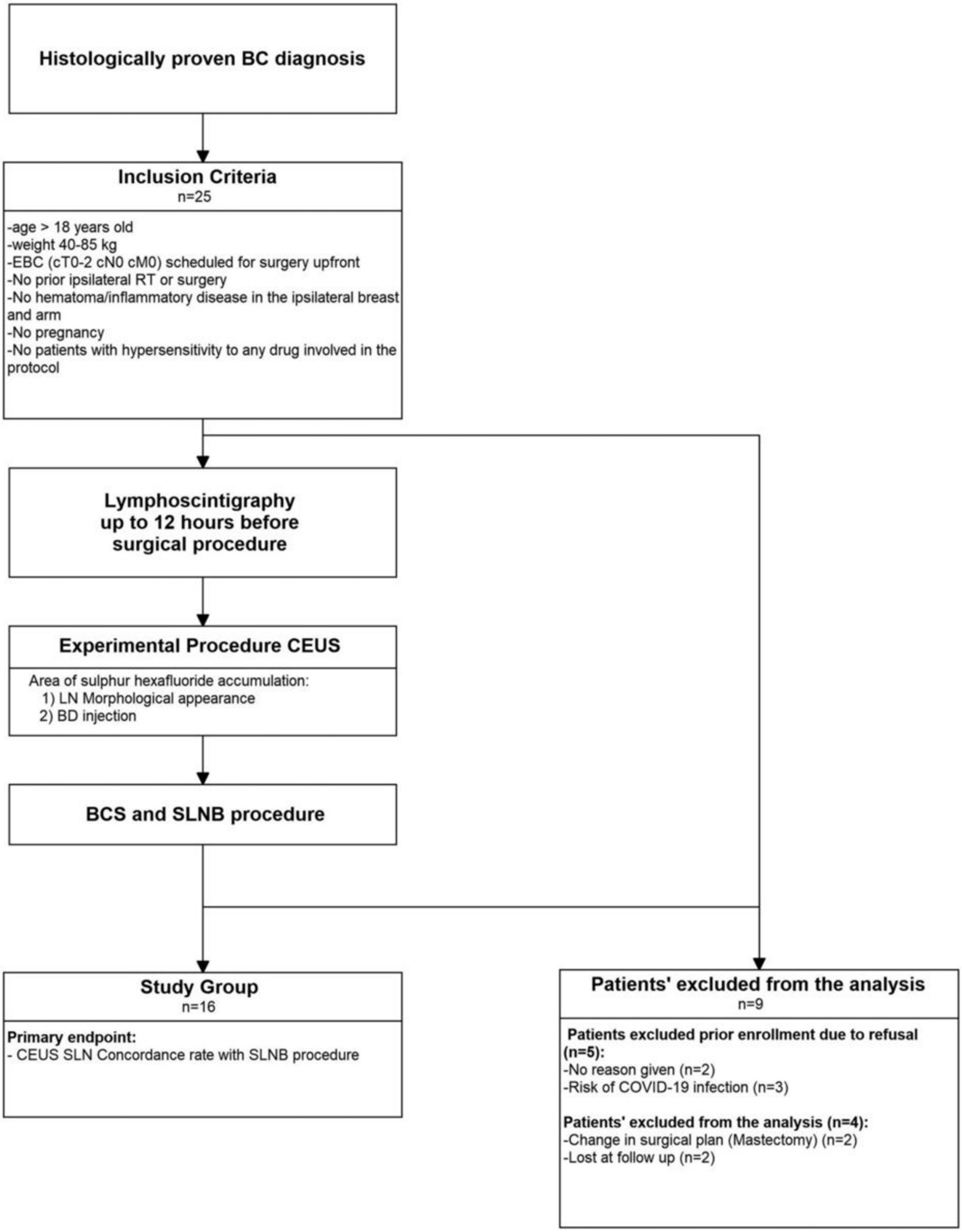

Figure 1. *Axillary contrast-enhancement ultraSound evaluation (AX-CES) 1.2020 study flowchart. BC: Breast cancer; BCS: breast-conserving surgery; BD: blue dye; CEUS: contrast-enhanced ultrasound; COVID-19: coronavirus disease 19; EBC: early breast cancer; LN: lymph node; RT: radiotherapy; SLN: sentinel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

were removed following the '10% rule' (all LNs with counts >10% of ex-vivo count of hottest LN should be removed), or if abnormal (18). After standard mapping, the number of LNs harvested with isotope, which were marked during CEUS SLN, were analyzed separately. Surgical theatre occupation time was recorded among all different stages of surgery: CEUS SLN procedure, BCS surgery, SLNB surgery, Frozen section SLNB.

Moreover, total inpatient stay was recorded. Patient re-evaluation occurred in the first, the second, and the thirtieth postoperative day to assess surgical complications, which were recorded according to the breast modified Clavien-Dindo classification (19). Pathological assessment included histological classification, 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 (ITC)] or macrometastatic (metastasis >2 mm). Expression of estrogen receptor, progesterone receptor and Ki67 protein was expressed as a percentage, and over-expression 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 (20).

Statistical analysis. All patient data were recorded in an Excel database (Microsoft, Redmond, WA, USA). Continuous variables are recorded as mean and interquartile range (IQR). Categorical variables are reported as numbers and percentage. SPSS statistical package version 23.0 was used (IBM Corp., Armonk, NY, USA).

CEUS SLN procedure appearance and standard SLN procedure frozen section were evaluated in comparison to the definitive staining of the standard mapping procedure. The assessment included the analysis of sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV). Continuous variables were compared between the procedures using the Mann–Whitney *U*-test, and for categorical variables, the Fisher exact test was applied. *p*-Values <0.05 were considered statistically significant.

Results

Baseline data. Among the 25 patients with BC considered for enrollment, five patients refused. In three patients the reason reported was the risk of COVID-19 infection, whereas two patients did not report any specifical reason. As mentioned earlier, the COVID-19 pandemic determined a steady difficulty in patient enrollment, affecting daily clinical practice in several aspects (21). After enrollment, four patients were excluded from the analysis. Change in surgical plan (mastectomy) occurred in two patients, and the other two patients were lost to follow up.

Therefore, 16 patients with BC who underwent the CEUS SLN procedure were included in the study. Table I summarizes the baseline characteristics. The mean age was 57.75 (40.50-75.75) years. Regarding histology, 12 (75.00%) cases were classified as invasive ductal BC, two (12.50%) as lobular BC, and two (12.50%) as other (one tubular BC and one case of medullary BC). Twelve (75.00%) cases were reported in the outer quadrant and four (25.00%) cases in the inner quadrant. Moreover, at final pathology, eight (50.00%) were described as multicentric/multifocal BC, and in 5 (31.25%), ductal carcinoma *in situ* (DCIS) was associated with invasive BC.

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 (6.20%)
Negative	7 (43.80%)
LN from standard mapping painted with BD (n=16), n (%)	
Yes	15 (87.50%)
No	1 (12.50%)

All continuous variables are reported as median and interquartile range (IQR), while categorical variables are reported as frequencies and percentages. BMI: Body mass index; DCIS: ductal carcinoma *in situ*; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; ITC: isolated tumor cells; LN: lymph node; PR: progesterone receptor; SLN: sentinel lymph node.

The median surgical theatre occupation time without CEUS was 123.24 min (111.50-134.50 min). BCS required

96.81 min (87.56-101.45 min), 78.55% of the total time, while SLNB with frozen section response required 35.43 min (15.76-48.23 min) (21.45% of the total occupation). The mean time of surgical occupation theatre spent waiting for frozen section results was 24.21 min (10.75-33.11 min) (19.64% of the total occupation). Therefore, a total of 387.86 min were spent in the surgical theatre waiting for frozen section results. The time spent in the surgical theatre waiting for the frozen section results was equivalent to the time spent for 3.91 patients that underwent to BCS and SLNB.

CEUS procedure. During surgery, the CEUS SLN procedure detected a total of 29 LNs among 16 patients, which were marked with blue dye. In all cases (100%), the CEUS SLN procedure was reported as technically successful and at least one LN was identified (median LNs 2). The mean procedure time recorded was 18.87 min, as shown in Figure 2, but the CEUS SLN procedure time reported in 1-5 cases was statistically significantly longer than the time reported for cases 11-16 (23.46 *vs*. 17.26 min; p=0.011).

After the CEUS SLN procedure, six (37.50%) LNs were defined pathological, which were not detectable using conventional US. No complications were reported after the CEUS SLN procedure. The definitive assessment of CEUS SLN pathology revealed SLNs' macrometastatic involvement in four (66.67%) of cases. The CEUS SLN procedure failed to identify two macrometastatic LNs. In both cases, due to the low disease burden and clinic-pathological features, axillary lymph node dissection (ALND) was not performed, according to the Z0011 study. In the first case a single macrometastatic LN was identified, and in the second case, two positive LNs were classified as macrometastatic out of four LNs collected.

SLN procedure. After the CEUS SLN procedure, standard mapping techniques were performed in the standard fashion. Isotope techniques detected 40 SLNs (median SLNs 2.5). When compared with the CEUS SLN procedure, a statistically significantly higher number of LNs were reported in the standard procedure (2.5 *vs*. 2, p=0.025).

Intraoperative assessment of standard mapping techniques revealed five (83.34%) macrometastatic SLNs and failed to identify one macrometastatic SLN. However, due to the low disease burden and clinical-pathological features, ALND was not performed according to the Z0011 study.

After surgery, in the definitive assessment, at least one macrometastatic LN was reported in six (37.50%) patients. Ten (62.50%) patients were classified as negative, three (18.70%) of which were patients with ITC or micrometastatic foci.

Concerning the CEUS SLN procedure, in 15 cases (93.75%) at least one harvested SLN with standard mapping technique was marked with blue dye and a total of 26 (65.00%) blue stained LNs were harvested during the standard mapping procedure. The definitive staining of blue dye CEUS SLNs revealed how in five (31.25%) patients at least one of the blue stained LNs was metastatic on pathological analysis.

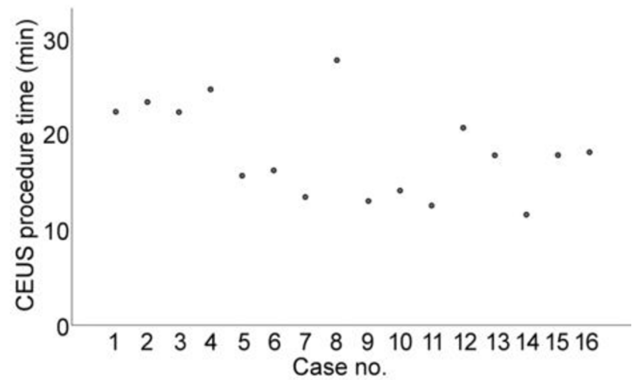

Figure 2. *Contrast-enhanced ultrasound (CEUS) sentinel lymph node (SLN) procedure time according to single procedure time. Time is reported in minutes of each CEUS SLN.*

Table II lists the results of the CEUS SLN procedure and intraoperative assessment of SLN standard mapping techniques when compared with the gold standard technique (definitive assessment of the SLN specimen). Table III presents the diagnostic performances (sensibility, specificity, PPV, and NPV) of both techniques.

When compared to frozen section intraoperative assessment, CEUS SLN demonstrated lower sensitivity (66.7% *vs*. 83.3%) and specificity (80% *vs*. 100%). VPN of CEUS SLN for predicting ALN status was 80.00%. Interestingly, as stated before, while CEUS failed preoperatively to detect SLN metastasis in two cases, no further axillary surgical procedure was planned in multidisciplinary treatment due to the lower disease burden.

Discussion

In AX-CES 1.2020, the CEUS SLN procedure represented a promising technique to enhance preoperative axillary status in the outpatient setting and potentially reduce surgical extent in the axilla with a potential sparing of SLNB procedure in selected patients.

The SLNB procedure embodied a pivotal step toward surgical de-escalation in EBC providing equivalent oncological outcomes of further axillary procedure, such as ALND (5, 6). However, while lower, the SLNB procedure is associated with the same complications of other axillary procedures, such as ALND, arm lymphedema (5%) and sensory loss (18% at 1 month) (22). In fact, even if limited, postoperative fibrosis, axillary nerve and lymphatics injury

Table II. *Metastatic status of sentinel lymph node (SLN) prediction using contrast-enhanced ultrasound (CEUS), or intraoperative assessment using standard mapping (injection of radioactive isotope, ^{99m}Tc-nanocolloid human serum albumin).*

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

ALN: Axillary lymph node.

Table III. *Diagnostic performance of the contrast-enhanced ultrasound (CEUS) procedure and frozen section compared to standard mapping (injection of radioactive isotope ^{99m}Tc-nanocolloid human serum albumin).*

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CEUS pathological appearance	66.70%	80.00%	66.70%	80.00%
Standard mapping macrometastasis in frozen section	83.30%	100.00%	100.00%	90.91%

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

are associated with detrimental effects on upper limb physical function when compared to patients who are spared from any axillary procedure (9). Many authors started to inquiry about the role of surgical axillary staging in EBC (8, 9, 23). In fact, in the last five years, growing evidence from ACOSOG Z0011, IBCSG 23.01, and SOUND trials, plus genomic analyses, shifted from an old lymphatics-center approach toward an holistic approach centered on clinical, genomic, and biomolecular characteristics in order to determine the patient tailored treatment where axillary status is not always required to determine adjuvant treatment (7, 10, 24, 25).

Under this perspective, SLNB, designed as one-fits-all procedure to discriminate node-negative from node-positive patients, demonstrated its intrinsic limitation for precise patient stratification. For instance, data from the SOUND study demonstrate how LN metastasis in low-risk patients occurs in 13.7% of cases, with a significant number of patients who will eventually undergo an invasive procedure without clear clinical benefit. In fact, in the SOUND study non-invasive axillary staging, such as US, did not determine any detrimental effect on 5-years OS, DFS, or further suggested adjuvant treatment when compared with SLNB standard-of-care (10).

Mammography plus conventional US are nowadays the minimum requirement for BC locoregional staging. Regarding axillary US, dimensional, morphological and doppler features are routinely combined to predict ALN status, with a sensitivity ranging in the literature from 24% to 94% (12, 13). In fact, while conventional US is effective for relevant nodal disease burden, failure has been reported in up to 30% of cases with detrimental effect on treatment planning (26). As expected in our analysis, six (37.50%) patients were misclassified as nodal negative during preoperative assessment, and four of them were correctly predicted as positive using the CEUS SLN procedure.

In fact, when compared with morphological US, CEUS SLN, owing to the contrast agent administration, may facilitate ALN staging by providing hybrid imaging that can easily differentiate the localization of lymphatic channel and isoechoic LNs from the axillary tissue (27). Moreover, when combined with morphological US, different enhancement models have been described in the literature with different ALN metastasis likelihood (17, 27). While in our analysis no formal assessment was carried out regarding LN enhancement patterns, in our series, six patients with at least one pathological LN after the CEUS SLN procedure were identified, with a putative benefit in terms of multidisciplinary cancer care and preoperative planning, providing a technical framework for CEUS-guided SLN core needle biopsy in preoperative setting.

Moreover, since its first description in 1998, preoperative and intraoperative US for BC entered in the clinical armamentarium of BC surgeons to localize breast masses, reduce the extent of healthy tissue removal in breast conserving surgery, and to perform locoregional anesthesia (28, 29). Regarding BC care, the great benefit of breast intraoperative US (IOUS) as an ancillary technique is a reduced margin involvement rate and improvement of overall cosmetic outcome at one year of follow up, as reported in the COBALT trial (30). Keeping in mind these benefits, many surgeons successfully approached breast US and became proficient in intraoperative and preoperative US application, providing a basis for the easy implementation of the CEUS SLN procedure into clinical practice by breast surgeons.

Under this perspective, in our study, the CEUS SLN procedure with a mean time procedure of 18.28 min represents an adaptable safe procedure. Without any local or systemic reaction observed in our series, the CEUS SLN procedure demonstrated its potential role as a non-invasive preoperative ALN assessment. Considering the sequelae of SLNB on the upper limp, potential clinical benefits of the CEUS SLN procedure included a reduction in surgical room occupancy and reduced arm disability (9, 10). In our series, the CEUS SLN procedure was technically successful in all patients in the study group, with a median number of two LNs identified during the procedure. The mean procedure time was consistent with data from the literature and, as expected, and a statistically significant reduction in retrieved SLNs was observed when comparing the CEUS SLN procedure with the traditional technique (31, 32). Although the performance of CEUS SLN procedure may be not considered apparently sub-optimal due to the lower number of SLNs enhanced, in our series, a concordance of 93.75% was obtained between techniques with at least one SLN harvested with traditional techniques and marked during CEUS.

In fact, while there is no clear consensus on the minimum number of harvested SLNs in EBC, removal of fewer SLNs is not associated with locoregional disease control failure, but is associated with a higher false negative rate. In clinical practice, the surgeon's experience plays a pivotal role in the final number of SLNs harvested during the surgical procedure, with high variability between surgeons (33). Despite a lower number of SLNs not being considered a contraindication, we believe that physician technique proficiency may have influenced our results. While a lower mean procedure time has been recorded in the last five patients of our series, it has been calculated that 25 procedures were needed to master this technique and larger series are needed to confirm our preliminary data (31).

While a lower number of LNs were reported in our series, the high concordance rate strengthens the validity of the CEUS SLN procedure in EBC and potentially in other different clinical settings as an ancillary technique for invasive and non-invasive axillary staging. Under this perspective, the CEUS SLN procedure may be used for several purposes in different clinical stages, as displayed in Figure 3, including risk factor stratification according to age, performance status, clinical stage, tumor biomolecular classification and possible genomic assay.

According to the stratification, the CEUS SLN procedure may be applied as non-invasive axillary staging in low-risk BC with potential savings in surgical room occupancy in T0-2 cN0 post-menopausal women, or patients with low performance status (4, 34). In our series, nine (56.25%) patients were older than 50 years old and they could eventually benefit from surgical axillary staging. Additionally, patients with high risk of axillary involvement (large, multicentric tumor, aggressive biology) may benefit from non-invasive axillary evaluation with the CEUS SLN procedure to reduce the risk of hidden disease burden that may benefit from primary systemic treatment (PST).

We acknowledge that several limitations may have influenced our results. First, the small sample size could have impacted our findings. However, our research was structured as a pilot study aimed at exploring the technical application and concordance rate of CEUS with standard mapping, and no power analysis was conducted. Both procedures (CEUS SLN procedure and traditional procedure) were performed in surgical theatre for safety reasons. A larger-scale study will be devised to validate our initial data in the outpatient setting. Another potential source of error may have been associated with the oncological characteristics of the patients included in the study. Due to the inclusion criteria, most enrolled patients may have been categorized as having a low risk of axillary involvement, and this may have influenced our results in terms of axillary involvement and thus sensitivity and specificity of CEUS SLN procedure. However, stringent clinical inclusion criteria was an informed choice made during the study design. Low risk EBC population with low risk of axillary involvement, as according to Z0011 criteria and the SOUND study, with a clear indication for upfront surgery, is currently one of the BC populations where studies on axillary surgical de-escalation are ongoing. While promising in other clinical settings (*e.g.*, dual tracer technique after PST), further investigations with larger cohorts should explore the role of CEUS SLN procedure in those specific populations.

In conclusion, despite these limitations, our preliminary data demonstrates how the CEUS SLN procedure may be a promising and safe technique to perform non-invasive assessment of the axilla, aiming at a safe axillary de-escalation in EBC. The addition of CEUS in the preoperative setting may represent an advantage due to its higher sensitivity and specificity, when compared to conventional US to estimate axillary disease burden. Further studies are needed to confirm this data and determine the clinical usefulness of the CEUS SLN procedure in different clinical setting as outpatient SLN core-needle biopsy, pre and post NACT locoregional staging, or axillary disease burden assessment.

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, Di Lorenzo Nicola. Formal analysis: Tacconi Federico, Chiara Adriana Pistolese

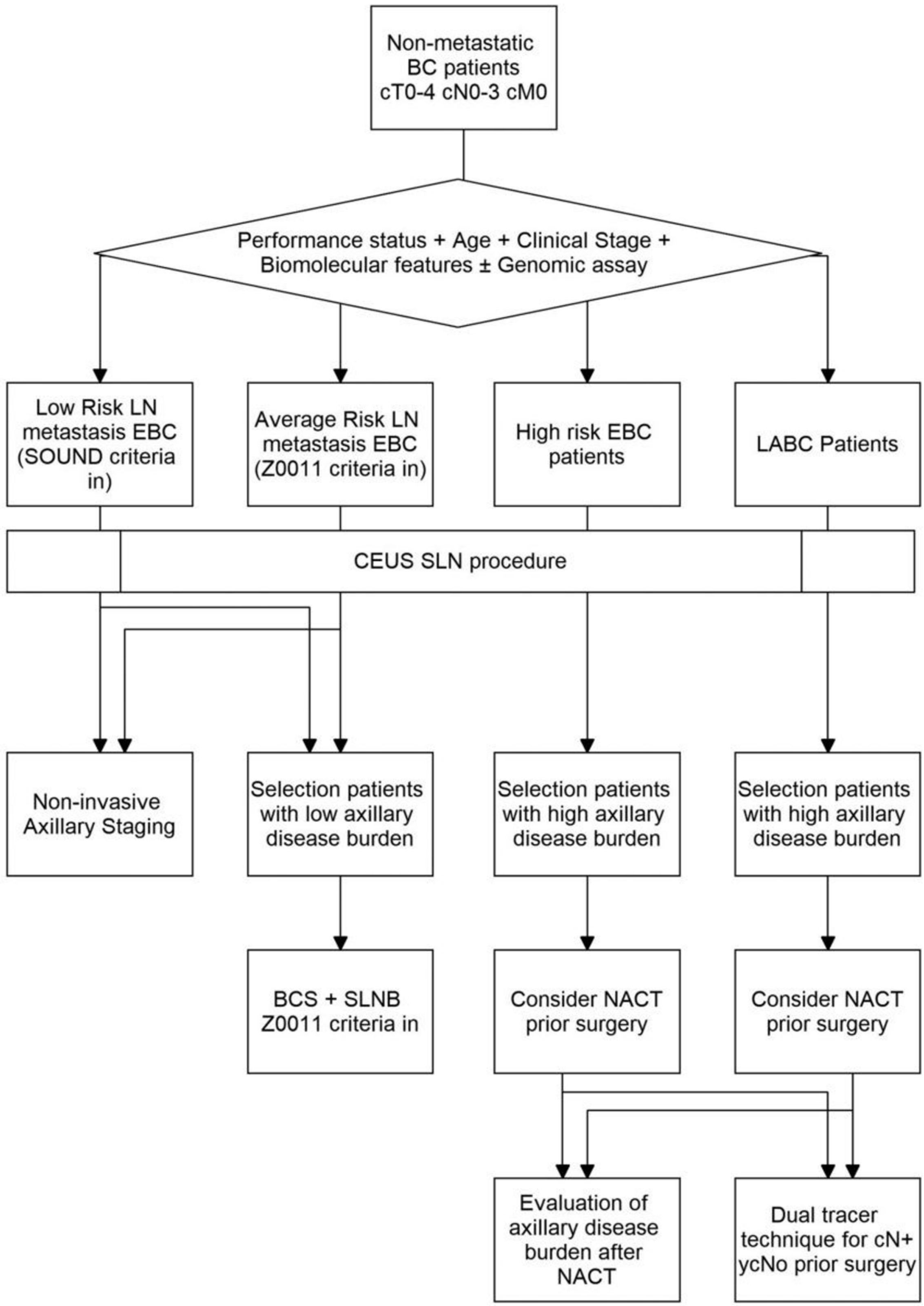

Figure 3. *Potential contrast-enhanced ultrasound (CEUS) application in non-invasive axillary staging and invasive axillary staging according to the age, performance status, clinical stage presentation (I-IIIC), and genomic risk of recurrence. BC: Breast cancer; LN: lymph node; EBC: early breast cancer; LABC: locally advanced breast cancer; SLNB: sentinel lymph node biopsy; NACT: neoadjuvant chemotherapy.*

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