Original Research

Endoscopic Ultrasound

OPEN

Identification of sentinel lymph nodes in esophageal cancer patients using contrast-enhanced EUS with peritumoral injections

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ABSTRACT

Objectives: The objective of this pilot study was to compare the performance of contrast-enhanced EUS (CE-EUS)–guided fine-needle aspiration (FNA) with EUS-FNA for lymph node (LN) staging in esophageal cancer.

Methods: Thirty-seven subjects with esophageal cancer undergoing EUS staging were enrolled, and 30 completed this institutional review board–approved study. A Prosound F75 US system (Hitachi Medical Systems, Tokyo, Japan) with harmonic contrast imaging software and GF-UCT180 curvilinear endoscope (Olympus, Tokyo, Japan) was utilized. All LNs identified by standard EUS were first noted. Sonazoid (dose: 1 mL; GE Healthcare, Oslo, Norway) was administered peritumorally, and all enhanced LNs were recorded. Fine-needle aspiration was performed on LNs considered suspicious by EUS alone, as well as LNs enhanced on CE-EUS. Performance of each modality was compared using FNA cytology as reference standard.

Results: A total of 132 LNs were detected with EUS, of which 59 showed enhancement on CE-EUS. Fifty-three LNs underwent FNA, and 22 LNs were determined to be malignant. Among the latter, 10 were considered suspicious by EUS, whereas the other 12 LNs underwent FNA only because of CE-EUS enhancement. Contrast-enhanced 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. Eight subjects (8/30 [27% of study total]) had nodal status upgraded by the addition of CE-EUS, which influenced LN staging and clinical management.

Conclusions: Fine-needle aspiration of LNs identified by CE-EUS may increase metastasis positive rate by ruling out LNs not associated with the tumor drainage pattern. In addition, CE-EUS seems to identify more metastatic LNs that would not be biopsied under the standard EUS criteria.

Key words: Esophageal cancer; Contrast-EUS; Sentinel lymph node; EUS; Fine needle biopsy

INTRODUCTION

Esophageal cancer is the eighth most common cancer and the sixth leading cause of death from cancer worldwide.[1] The incidence of esophageal cancer has been rising in Western countries over the past decades.[2–4] In the United States, it is estimated that 20,640 esophageal cancer cases are diagnosed each year, and 16,410 deaths are expected.[4] Esophageal cancer is an aggressive disease associated with a very poor prognosis (5-year survival rate of 20%), because most patients present with advanced tumor stage at the time of diagnosis.[5–7]

Detection of subclinical malignancy in draining lymph nodes (LNs) has tremendous importance in the management of a variety of malignancies including esophageal cancer, but also melanoma and breast, colon, and other cancers.[8–12] Establishing LN involvement is vital to determine both the treatment and prognosis of esophageal cancer.[13] The most important LN to evaluate is the sentinel lymph node (SLN), which is defined as the first node in the regional lymphatic system to receive afferent drainage through lymphatic channels from the primary tumor.[14–17]

Although grayscale ultrasound, color Doppler, and pulsed Doppler have been used alone or in combination to assess LNs for the presence of metastases, conventional ultrasound cannot be used for lymphatic mapping (ie, to identify a tumor's SLNs), because mapping requires administration of a tracer (*e.g.*, dye or radioisotope).[15,16,18–20] Microbubble-based ultrasound contrast agents (UCAs) have been extensively used for enhancement of vascularity and perfusion.[21] Our group and others have confirmed that SLNs can be identified with contrast-enhanced ultrasound (CEUS) following subcutaneous or peritumoral injections of an UCA around tumor sites in several animal species.[16–20] We have subsequently demonstrated the safety of this approach (termed lymphosonography) in healthy volunteers[15] and transitioned

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Endoscopic Ultrasound (2023) 12:4

Received: 8 July 2023; **Accepted:** 12 April 2023

Published online: 13 September 2023

http://dx.doi.org/10.1097/eus.0000000000000001

this work to a clinical trial using Sonazoid (GE Healthcare, Oslo, Norway) for SLN identification in patients with breast cancer (NCT# 02652923).

The objective of this pilot study was to compare the performance of contrast-enhanced EUS-FNA (CE-EUS-FNA) for SLN identification in patients scheduled to undergo EUS-FNA for nodal staging of their esophageal cancer. Our hypothesis is that by injecting an UCA into the peritumoral tissues and following its uptake in the lymphatic channels using CE-EUS, it will be possible to identify the specific location and the number of SLNs draining a given esophageal cancer.

MATERIALS AND METHODS

Thirty-seven patients newly diagnosed with esophageal cancer who were scheduled for an EUS-FNA procedure for tumor and nodal staging were enrolled in this prospective pilot study from October 2018 to April 2022. All enrolled subjects provided written informed consent. The study was approved by the university's institutional review board, as well as the US Food and Drug Administration (IND no. 127419), and was compliant with the Health Insurance Portability and Accountability Act. The full protocol and statistical analysis plan available are listed under NCT# 03578224.

The inclusion criteria were subjects older than 18 years newly diagnosed with esophageal cancer who were scheduled for EUS-FNA procedure for tumor and nodal staging. The exclusion criteria were female subjects who were pregnant or breastfeeding, subjects with other primary cancers requiring systemic treatment, subjects with known hypersensitivity or allergy to any component of Sonazoid, and subjects who were medically unstable.

A Prosound F75 US system (Hitachi Medical Systems, Tokyo, Japan) with harmonic contrast imaging software and a GF-UCT180 curvilinear endoscope (Olympus, Tokyo, Japan) was utilized for both EUS and CE-EUS examinations. Figure 1 shows the scanner display and the US probe used in the study. The subjects underwent their standard EUS where the tumor was evaluated to determine the location, size, and invasion of the surrounding tissues. The standard EUS was used to identify the regional LNs and determine their level of suspiciousness (*e.g.,* enlarged size >10 mm, round shape, and the absence of hilar characteristics). All LNs identified had their measurements, location, and level of suspiciousness recorded.

Afterward, to perform the CEUS examination, a UCA was administered endoscopically peritumorally in 4 locations equally distant from each other in 0.25-mL increments for a total of 1.0 mL using a 23-gauge Carr-Locke injection needle. The UCA used in this study was Sonazoid (GE Healthcare), consisting of perflubutane microbubbles (mean diameter between 2.4 and 3.5 μm) stabilized by a phospholipid shell.[22] This UCA was selected based on the prior experience of our group and others indicating that the reticuloendothelial phase of this UCA makes it the best choice for this application.[15,17] Before administration, the single-use vial of 16 μL of Sonazoid was reconstituted with 2.0 mL of sterile water according to the manufacturer's instructions. Once reconstituted, the agent was used immediately.

Contrast-enhanced EUS of the LNs previously identified was performed starting immediately after UCA injection to determine the uptake of microbubbles. The EUS probe was moved around the esophagus to determine if there were other LNs that were not observed during the standard EUS that had uptake of UCA. The mean time of the CE-EUS examination was approximately 20 minutes. Of note, this was far shorter than the washout time of Sonazoid previously reported in breast LNs (retention >4 hours postinjection).[15] Nonlinear harmonic imaging mode (transmitting/receiving at 4.7/9.4 MHz) was used to visualize migration of the UCA from the tumor to any associated LNs. Scanning parameters, such as acoustic output power, focal zone placement, depth, and so on, were optimized on a case-by-case basis, albeit with the acoustic output power kept low (mechanical index <0.2) to minimize bubble destruction. The locations of all LNs with UCA enhancement were recorded. Digital clips and still images of the CEUS findings were also acquired for all cases.

Highly suspicious nodes (*e.g.,* enlarged size, round shape, and the absence of hilar characteristics) by EUS and all enhancing SLNs identified by CEUS underwent an FNA biopsy accessible based on the discretion of the attending physician. Lymph nodes located behind the tumor, organs, and/or cardiovascular structures, as well as beyond reachable LNs, were considered not amenable to FNA, and a clinical decision was made to not perform a biopsy on these LNs. Following EUS and CEUS assessments, EUS-guided FNA biopsy was performed on all accessible nodes (originally identified nodes considered suspicious by the gastroenterologists as well as all SLNs identified by CEUS-EUS) using a 25-gauge needle inserted through the working channel of the endoscope and advanced through the gut wall into the suspicious node under EUS guidance. The FNA of nodes was not conducted if the biopsy needle had to

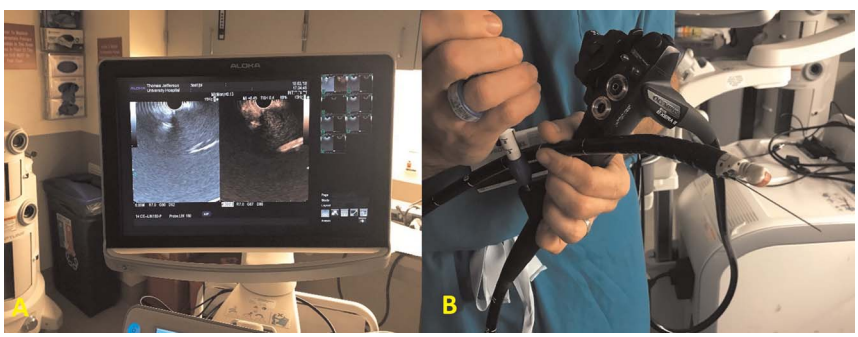

Figure 1. Imaging apparatus. A, Dual-imaging mode display for grayscale and harmonic contrast imaging. B, EUS probe with biopsy channel.

pass through the tumor, large blood vessels, or otherwise inaccessible tissues. The aspirate of the specimen was placed on a glass slide and processed with a Diff-Quik stain (Fischer Scientific, Hampton, NH, USA) and read by an on-site cytologist for the presence of tumor cells and also sent for histopathologic evaluation. The histopathologic findings were used as a reference standard for comparison of the EUS and CE-EUS results.

The data acquired with EUS and CE-EUS were compared with each other and with the pathology findings using McNemar test. All tests were performed using Prism 9.3.1 (GraphPad Software, San Diego, California), with $P < 0.05$ indicating statistical significance.

RESULTS

From the 37 subjects enrolled in the study, 30 subjects completed the study and had their data analyzed. Seven subjects had to be excluded from the study: 3 subjects had esophageal stricture related to the tumor extension that precluded the passage of the EUS endoscope, 2 subjects had no tumor identified during the procedure, 1 subject had their tumor excised at the time of the procedure without LN evaluation, and 1 subject did not have the CE-EUS done due to equipment failure. Figure 2 shows a diagram of subject enrollment and procedures.

The mean age of the 30 subjects who completed the study was 66 years (range, 43–90 years); 26 subjects (87%) were male, and 4 subjects (13%) were female. Twenty-four subjects (80%) were diagnosed with adenocarcinoma, and 6 subjects (20%) were diagnosed with squamous cell carcinoma. The tumor staging was T1a (n = 2 [7%]), T1b (n = 4 [13%]), T2 (n = 5 [17%]), and T3 (n = 19 [63%]). Table 1 shows a summary of the subjects' demographics, tumor type, and staging.

No adverse events were observed following peritumoral injection of Sonazoid under EUS guidance. The standard EUS identified a total of 132 LNs, from which 35 LNs (27%) were considered to be high risk or abnormal by standard EUS criteria, and 97 LNs (73%) were considered to be low risk or normal by the standard EUS. That translates to the fact that clinically from the 132 LNs identified only 35 LNs that were considered to be high risk would undergo FNA as part of the standard of care.

Contrast-enhanced EUS identified 59 SLNs of the 132 LNs identified with standard EUS. When the LNs identified with CE-EUS were divided using the standard EUS criteria for high risk/low risk, 17 SLNs identified with CE-EUS were part of the group of regional LNs that were considered high risk by standard EUS (48.6% [17 CE-EUS/35 EUS]), whereas additional 42 SLNs identified with CE-EUS were part of the group of regional LNs that were considered low risk by standard EUS (43.3% [42 CE-EUS/97 EUS]).

A total of 53 LNs that were identified during the study underwent FNA, with 22 LNs considered to be high risk by standard EUS. The other 17 LNs identified as high risk by standard EUS could not undergo FNA because of their location. Contrast-enhanced EUS

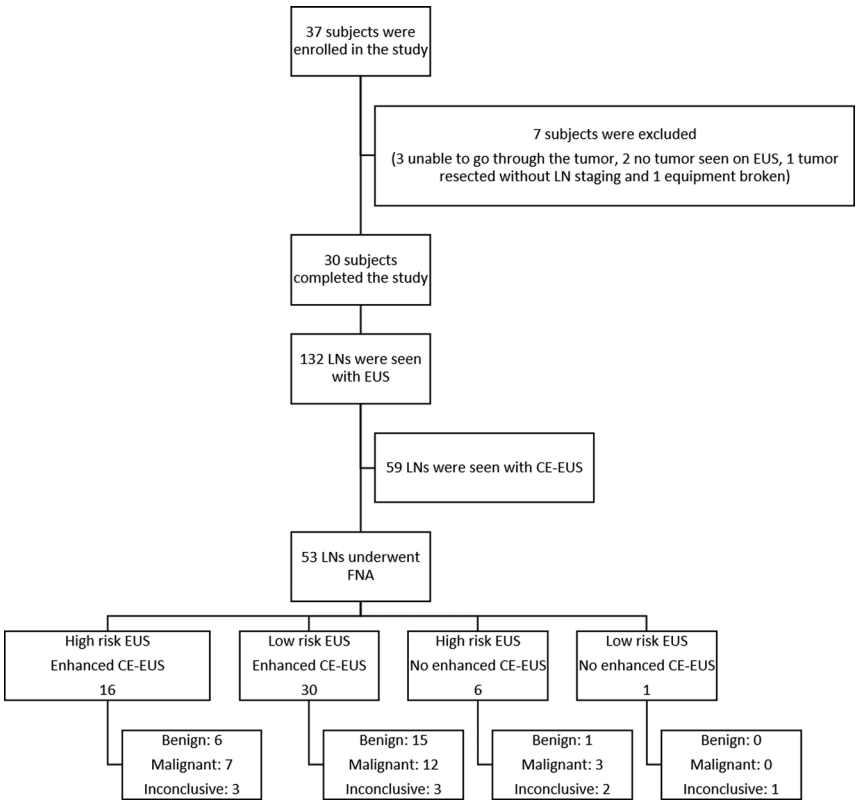

Figure 2. Enrollment diagram. CE-EUS, contrast-enhanced EUS; LNs, lymph nodes; FNA, fine-needle aspiration.

Table 1
Study demographics.

Subjects	30 (100%)
Sex	
Female	4 (13%)
Male	26 (87%)
Mean age, y	66 (range, 43–90)
Tumor type	
Adenocarcinoma	24 (80%)
Squamous cell carcinoma	6 (20%)
Tumor staging	
T1a	2 (7%)
T1b	4 (13%)
T2	5 (17%)
T3	19 (63%)

identified 46 LNs of the 53 LNs that underwent FNA. The remaining 13 LNs that were identified with CE-EUS could not undergo FNA because of their location. That indicates that with the use of CE-EUS additional 31 LNs that were considered high risk by standard EUS underwent FNA.

The cytological results from the LNs that underwent FNA showed that among the 53 LNs 22 were determined to be benign, and 22 were determined to be malignant, whereas the status of 9 LNs was determined as inconclusive because of lack of sample material.

Figure 3 shows an example of an LN that was determined to be benign by FNA and was considered low risk by EUS but showed enhancement on CE-EUS, indicating it was an SLN. Figure 4 shows an example of an LN that was determined to be malignant by FNA. Nonetheless, it was considered low risk by EUS, but showed enhancement on CE-EUS.

Among the 22 LNs that were malignant, only 10 were considered to be high risk by standard EUS, indicating that 55% of the FNA-confirmed malignant LNs would not have undergone an FNA in the clinical setting without CE-EUS, which indicates that in these cases without the use of CE-EUS the staging of their tumor would have been understated, affecting treatment choice and subsequently prognosis.

Overall, CE-EUS identified 59 nodes, of which 19 were conclusively diagnosed as containing metastatic disease (cytological yield of 32.3%). This was similar to EUS alone (10 of 35 nodes, cytological yield of 28.6%, $P = 0.25$). However, CE-EUS identified 19 LNs from the FNA-confirmed malignant 22 LNs, including the 12 LNs that the standard EUS considered to be low risk. Consequently, the rate of metastatic node identification for the standard EUS was 45% (10/22 LNs), and for CE-EUS, it was 86% of (19/22 LNs), which showed a statistically significant difference ($P = 0.008$). Table 2 shows a summary of the findings.

All LNs identified were measured as the clinical standard of care currently in use where LNs >10.0 mm in diameter are considered

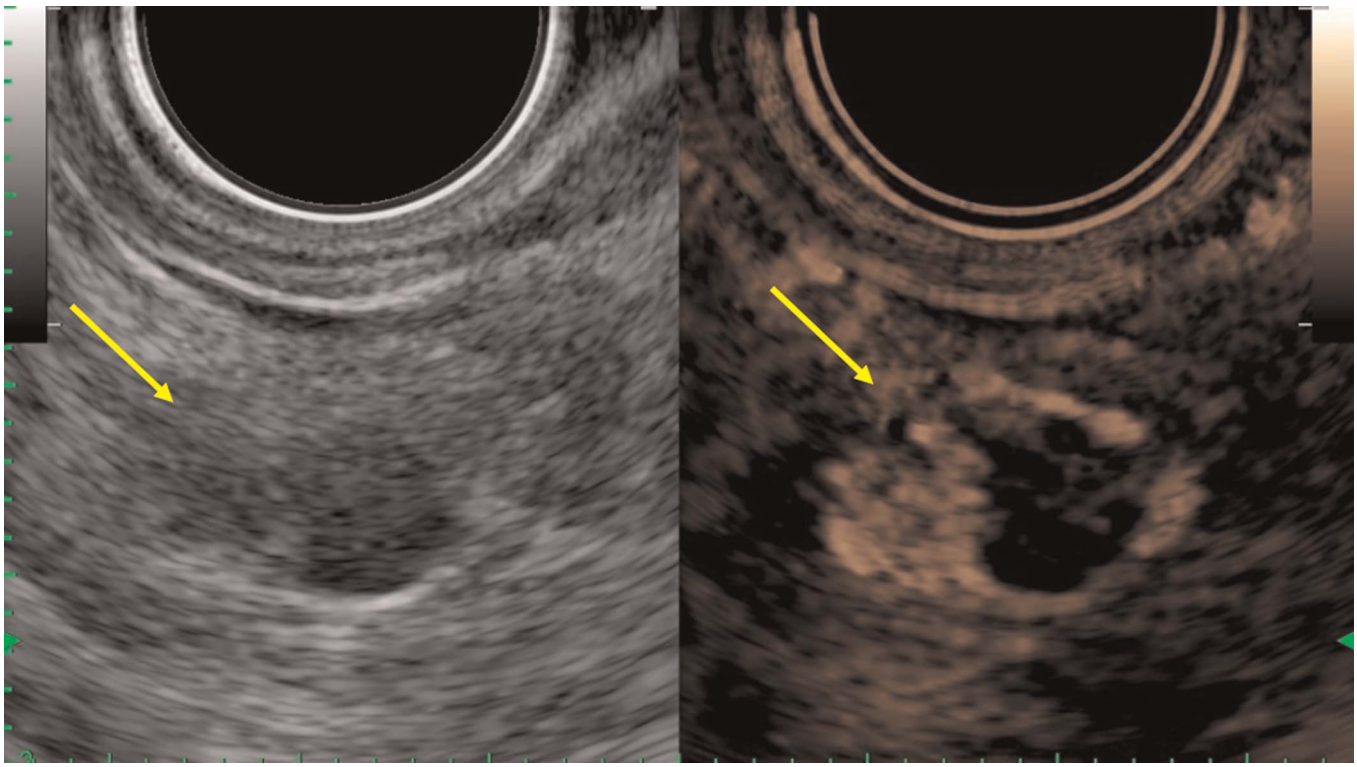

Figure 3. Dual-image B-mode and CEUS of the LN (arrow) in an example of a benign study case. The subject is a 68-year-old male patient diagnosed with an esophageal adenocarcinoma. EUS identified the LN, which was determined to be low risk because of its size, 1.0 × 0.5 cm. CE-EUS showed enhancement of the LN. The LN underwent FNA, which was determined to be negative for metastatic disease. CEUS, contrast-enhanced ultrasound; FNA, fine-needle aspiration; LN, lymph node.

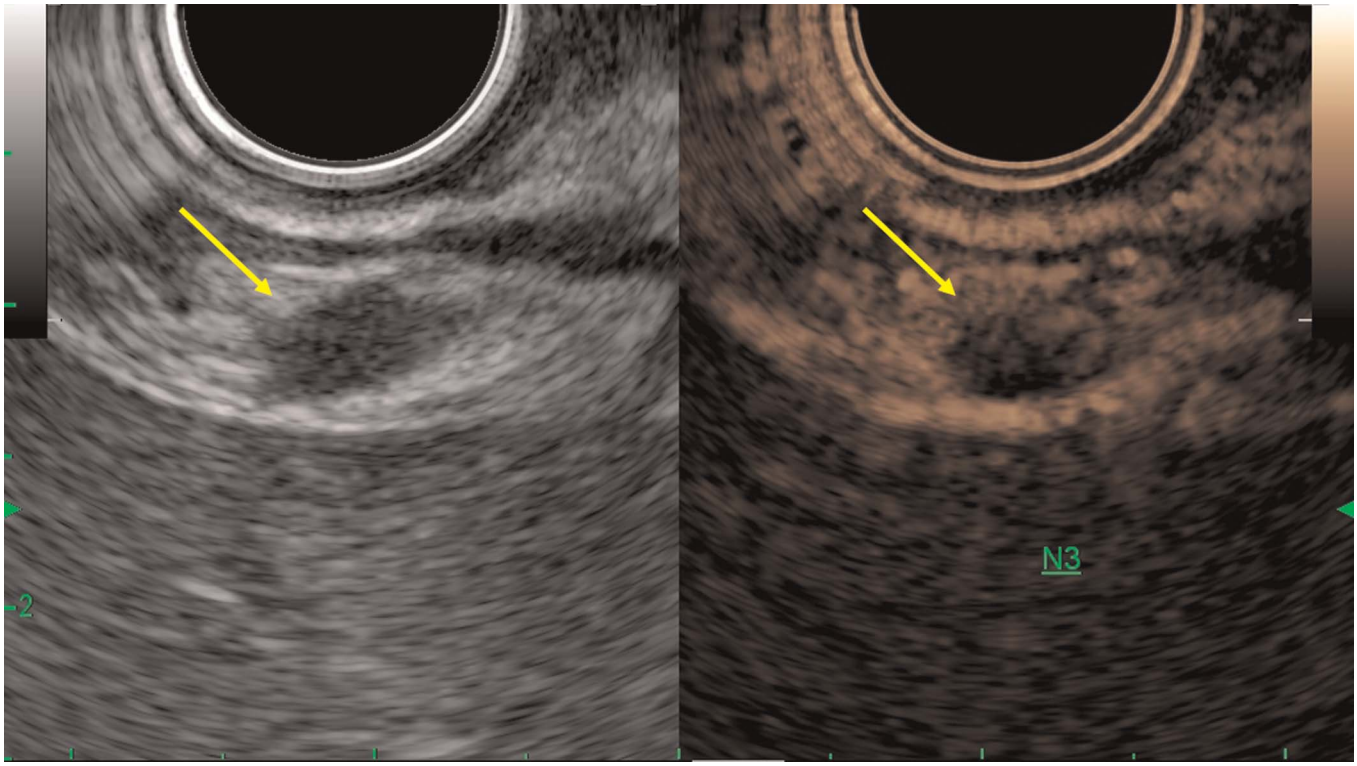

Figure 4. Dual-image B-mode and CEUS of the LN (arrow) in an example of a malignant study case. The subject is a 74-year-old male patient diagnosed with an esophageal adenocarcinoma. EUS identified the LN, which was determined to be low risk because of its size, 0.6 × 0.4 cm. CE-EUS showed enhancement of the LN. The LN underwent FNA, which was determined to be positive for metastatic disease. CE-EUS, contrast-enhanced EUS; CEUS, contrast-enhanced ultrasound; LN lymph node.

to be suspicious or high risk and selected for FNA when amenable. The mean size from the total number of LNs identified was 8.7 mm (range, 2.0–39.5 mm). The 22 LNs that pathology determined to be malignant had a mean size of 8.3 mm (range, 4.0–13.6 mm), where only 5 of 22 LNs were >10.0 mm. Therefore, only these 5 LNs would undergo FNA by using the standard of care, indicating that size alone is not an accurate way to identify LNs with metastatic disease. Table 3 shows a summary of LN sizes.

When the data were analyzed on a subject-by-subject basis, 8 subjects had their nodal status upgraded by the addition of CE-EUS, which translated to 27% of the total of subjects who completed the study (8/30). That means that the standard EUS determined the LN as low risk, CE-EUS showed enhancement in the same LNs, and FNA determined the LNs as being malignant; this directly influenced and changed the course of therapy for these subjects.

DISCUSSION

Evaluation of regional LNs is essential for patients with esophageal cancers to select the appropriate treatment and predict the patient's

Table 2

Study results on nodal burden.

	Enhanced CE-EUS (n = 59)	Nonenhanced CE-EUS (n = 73)
High-risk EUS (n = 35/total EUS n = 132)	17	18
FNA (n = 22/total FNA n = 53)	16	6
Benign	6	1
Malignant	7	3
Inconclusive	3	2
Low-risk EUS (n = 97/total EUS n = 132)	42	55
FNA (n = 31/total FNA n = 53)	30	1
Benign	15	0
Malignant	12	0
Inconclusive	3	1

CE-EUS, contrast-enhanced EUS; FNA, fine-needle aspiration.

Table 3

Measurements of LNs.

	No. of LNs	LN mean size, mm	LN minimal size, mm	LN maximum size, mm
Total	132	8.7	2.0	39.5
Non-FNA	79	8.2	2.0	39.5
FNA—malignant	22	8.3	4.0	13.6
FNA—benign	22	10.7	4.9	19.0
FNA—inconclusive	9	10.4	3.6	16.9

FNA, fine-needle aspiration; LN, lymph node.

prognosis. EUS has been used extensively in the detection of periesophageal lymphadenopathy and guided FNA cytology for distinguishing metastatic and benign LNs in terms of staging and clinical decision-making. However, currently, EUS can visualize only the regional periesophageal LNs that may not be true SLNs by definition. In addition, EUS provides no identification of nodal drainage patterns, because of the lack of a lymphatic tracer. Therefore, the selection of LNs for FNA biopsy can be challenging.

As standard clinical practice, patients newly diagnosed with esophageal cancer without evidence of distant metastasis (on computed tomography) undergo an EUS (as standard of care) for better T and N staging and assessment of locoregional disease.[23] Because the stage is strongly associated with prognosis, accurate clinical staging is essential for treatment planning. Superficial lesions without nodal involvement may be treated with endoscopic intervention.[24] Conversely, advanced locoregional disease may require neoadjuvant chemotherapy before surgery or be entirely unresectable.[23]

Results of prospective and retrospective studies of EUS-guided unenhanced biopsy in esophageal cancer have shown relatively poor accuracy rates for nodal staging (38%–51%).[25,26] Because of the important prognostic implications of nodal involvement and the potential impact on selecting patients for neoadjuvant therapy, it is essential to improve on the modest accuracy rates of EUS imaging in nodal staging. EUS remains the clinical standard for staging of these patients before surgery, primarily because alternative nodal imaging approaches are not feasible in esophageal cancer. Although SLN detection via existing lymphatic mapping agents such as blue dye and radioisotopes provides an accurate interrogation of SLN status during surgical lymphadenectomy (pooled accuracy of 85%), these techniques are not translatable to EUS because of the need for visual tracking and high spatial resolution in LN sampling.[27] As a result, LN evaluation in esophageal cancer is frequently performed via FNA of all accessible and suspicious LNs.[28] Because 95% of involved LNs in esophageal cancer are within 3 cm of the primary tumor, EUS-FNA is preferred over computed tomography–FNA, because of limitations of localization from the shine-through effect.[29–31] Of note, criteria for the stratification of patients at low risk or ultrahigh risk for nodal involvement for whom routine FNA may be omitted are currently evolving.[32]

The UCA used in this study (Sonazoid) is commercially available for characterization of liver lesions in Japan, South Korea, China, and Norway (for intravenous injection only), but not currently approved by the European Union or US regulatory agencies.[21] Although other UCAs are approved (for intravenous applications) in these countries, it is important to point out that our preclinical experience has shown that Sonazoid is superior for lymphatic imaging compared with other commercial agents.[17] This has been attributed to the surface charge and hydrogenated egg phosphatidylserine shell components in Sonazoid, which cause Kupffer cell uptake.[33]

This study shows that EUS-guided FNA biopsy yielded a rate of metastatic node identification of 45% (10/22) of the regional LNs, whereas CEUS-guided biopsy yielded a rate of metastatic node identification of 86% (19/22) of contrast-enhanced SLNs. Most importantly, 8 subjects (27% of study total) had their nodal status upgraded with the addition of CE-EUS findings, in which the standard EUS determined the LN as low risk, whereas CE-EUS showed enhancement in the same LNs, and FNA determined the LNs as being malignant, which was directly influencing and altering their clinical management and future course of therapy. These findings highlight the current shortcomings of EUS nodal staging in esophageal cancer and the important clinical improvements that can be achieved even by modestly improving SLN identification. To our best knowledge, this pilot study is the first to demonstrate the safety and feasibility of delineating the specific location and number of SLNs draining esophageal cancer by injecting an UCA into the peritumoral tissues and following its uptake in the lymphatic channels using EUS.

There are some limitations to this pilot study, including the small number of cases enrolled, which means we cannot draw substantial statistical conclusion for the efficacy and clinical outcome. It should be pointed out that FNA with cytopathology can have inherent limitations due to incomplete sampling and inconclusive cytological reading. Because of the tumor's location within the esophagus, massage of the injection site cannot be conducted, unlike more superficial areas (such as breast tumor),[15] which may affect the UCA absorption and drainage. Because of the nature of esophageal cancer, many LNs that can be visualized and potentially identified as suspicious may not necessarily be amenable to FNA, because of their location behind the tumor, organs, and/or cardiovascular structures, as well as unreachable LNs. Finally, per the authors' protocol, a biopsy was not performed on the majority of nodes identified as not suspicious on either standard EUS or CE-EUS. This decision was made in conjunction with the clinical team to prioritize patient's care. Hence, the diagnostic performance (in particular, the negative predictive value) of both modes is artificially low due to relatively few pathologically confirmed negative cases. The image quality of harmonic imaging available on clinical EUS scanners does not match with the image quality observed in cart-based systems. However, a partnership between Olympus and Bracco was recently announced to resolve this issue.[34]

In conclusion, CE-EUS of SLN detection via lymphosonography is a safe and feasible technique for esophageal cancer nodal staging. Our preliminary results suggest CE-EUS may identify SLN with

metastatic deposits that would not normally be biopsied under standard EUS criteria. Contrast-enhanced EUS seems to have the potential to improve SLN identification and in esophageal cancer patients by offering a more reliable tumor staging and thereby improving clinical decision-making.

Funding

This work was supported by the National Institutes of Health (grant R21 CA218946).

Conflicts of Interest

J.-B.L.: contrast agent support GE. J.R.E.: patent, grant, and equipment support from GE. F.F.: patent, grant, and equipment support from Canon; equipment and drug support GE; drug support from Bracco and Lantheus; consultant to Exact Therapeutics. C.E.W.: clinical consultant to Bracco Diagnostics USA and speakers' bureau at Canon Medical Systems USA. AS: consultant to Fujifilm, Conmed, Lumendi, and Olympus. The other authors declare that they have no financial conflict of interest with regard to the content of this report.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Liu Ji-Bin, Priscilla Machado, John R. Eisenbrey and Flemming Forsberg. The first draft of the manuscript was written by Ji-Bin Liu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Rafiemanesh H, Mehtarpour M, Khani F, et al. Epidemiology, incidence and mortality of lung cancer and their relationship with the development index in the world. *J Thorac Dis* 2016;8(6):1094–1102.
2. Blot WJ, Devesa SS, Kneller RW, Fraumeni JF. Rising incidence of adenocarcinoma of the esophagus and ngastric cardia. *JAMA* 1991; 265(10):1287–1289.
3. Pera M, Cameron AJ, Trastek VF, Carpenter HA, Zinsmeister AR. Increasing incidence of adenocarcinoma of the esophagus and esophagogastric junction. *Gastroenterology* 1993;104(2):510–513.
4. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* 2022;72:7–33.
5. Forastiere AA, Heitmiller RF, Kleinberg L. Multimodality therapy for esophageal cancer. *Chest* 1997;112(4):195–200.
6. Pommier RF, Vetto JT, Ferris BL, Wilmarth TJ. Relationships between operative approaches and outcomes in esophageal cancer. *Am J Surg* 1998;175(5):422–425.
7. Roder JD, Busch R, Stein HJ, Fink U, Siewert JR. Ratio of invaded to removed lymph nodes as a predictor of survival in squamous cell carcinoma of the oesophagus. *Br J Surg* 1994;81(3):410–413.
8. Albertini JJ, Lyman GH, Cox C, et al. Lymphatic mapping and sentinel node biopsy in the patient with breast cancer. *JAMA* 1996;276(22): 1818–1822.
9. Alazraki NP, Styblo T, Grant SF, Cohen C, Larsen T, Aarsvold JN, eds. *Sentinel Node Staging of Early Breast Cancer Using Lymphoscintigraphy and the Intraoperative Gamma-Detecting Probe*. USA: Elsevier; 2000.
10. Morton DL, Hoon DSB, Cochran AJ, et al. Lymphatic mapping and sentinel lymphadenectomy for early-stage melanoma: therapeutic utility and implications of nodal microanatomy and molecular staging for improving the accuracy of detection of nodal micrometastases. *Ann Surg* 2003;238(4):538–550.
11. Yudd AP, Kempf JS, Goydos JS, Stahl TJ, Feinstein RS. Use of sentinel node lymphoscintigraphy in malignant melanoma. *Radiographics* 1999;19(2): 343–356.
12. Weisberg NK, Bertagnolli MM, Becker DS. Combined sentinel lymphadenectomy and Mohs micrographic surgery for high-risk cutaneous squamous cell carcinoma. *J Am Acad Dermatol* 2000;43(3):483–488.
13. Altorki NK, Zhou XK, Stiles B, et al. Total number of resected lymph nodes predicts survival in esophageal cancer. *Ann Surg* 2008;248(2):221–226.
14. Morton DL, Chan AD, eds. *The Concept of Sentinel Node Localization: How It Started*. USA: Elsevier; 2000.
15. Machado P, Stanczak M, Liu JB, et al. Subdermal ultrasound contrast agent injection for sentinel lymph node identification: an analysis of safety and contrast agent dose in healthy volunteers. *J Ultrasound Med* 2018;37(7): 1611–1620.
16. Goldberg BB, Merton DA, Liu J-B, et al. Contrast-enhanced ultrasound imaging of sentinel lymph nodes after peritumoral administration of Sonazoid in a melanoma tumor animal model. *J Ultrasound Med* 2011; 30(4):441–453.
17. Goldberg BB, Merton DA, Liu J-B, Murphy G, Forsberg F. Contrast-enhanced sonographic imaging of lymphatic channels and sentinel lymph nodes. *J Ultrasound Med* 2005;24(7):953–965.
18. Goldberg BB, Merton DA, Liu J-B, et al. Sentinel lymph nodes in a swine model with melanoma: contrast-enhanced lymphatic US. *Radiology* 2004; 230(3):727–734.
19. Liu J-B, Merton DA, Berger AC, et al. Contrast-enhanced sonography for detection of secondary lymph nodes in a melanoma tumor animal model. *J Ultrasound Med* 2014;33(6):939–947.
20. Curry JM, Ezzat WH, Merton DA, et al. Thyroid lymphosonography: a novel method for evaluating lymphatic drainage. *Ann Otol Rhinol Laryngol* 2009;118(9):645–650.
21. Lyshchik A. *Specialty Imaging: Fundamentals of CEUS E-Book*. USA: Elsevier Health Sciences; 2019.
22. Sontum PC. Physicochemical characteristics of Sonazoid™, a new contrast agent for ultrasound imaging. *Ultrasound Med Biol* 2008;34(5): 824–833.
23. Amin MB, Edge SB, Greene FL. *AJCC Cancer Staging Manual*. New York: Springer; 2017.
24. Yamada M, Oda I, Nonaka S, et al. Long-term outcome of endoscopic resection of superficial adenocarcinoma of the esophagogastric junction. *Endoscopy* 2013;45(12):992–996.
25. Kalha I, Kaw M, Fukami N, et al. The accuracy of endoscopic ultrasound for restaging esophageal carcinoma after chemoradiation therapy. *Cancer* 2004;101(5):940–947.
26. Laterza E, de Manzoni G, Guglielmi A, Rodella L, Tedesco P, Cordiano C. Endoscopic ultrasonography in the staging of esophageal carcinoma after preoperative radiotherapy and chemotherapy. *Ann Thorac Surg* 1999; 67(5):1466–1469.
27. Filip B, Scarpa M, Cavallin F, Alfieri R, Cagol M, Castoro C. Minimally invasive surgery for esophageal cancer: a review on sentinel node concept. *Surg Endosc* 2014;28(4):1238–1249.
28. Vazquez-Sequeiros E, Wiersema MJ, Clain JE, et al. Impact of lymph node staging on therapy of esophageal carcinoma. *Gastroenterology* 2003;125(6): 1626–1635.
29. Balalis GL, Thompson SK. Sentinel lymph node biopsy in esophageal cancer: an essential step towards individualized care. *Ann Surg Innov Res* 2014;8(1):1–5.
30. Van de Ven C, de Leyn P, Coosemans W, van Raemdonck D, Lerut T. Three-field lymphadenectomy and pattern of lymph node spread in T3 adenocarcinoma of the distal esophagus and the gastro-esophageal junction. *Eur J Cardiothorac Surg* 1999;15(6):769–773.
31. Gretschel S, Bembenek A, Hünerbein M, Dresel S, Schneider W, Schlag PM. Efficacy of different technical procedures for sentinel lymph node biopsy in gastric cancer staging. *Ann Surg Oncol* 2007;14(7):2028–2035.
32. Vazquez-Sequeiros E, Levy MJ, Clain JE, et al. Routine vs. selective EUS-guided FNA approach for preoperative nodal staging of esophageal carcinoma. *Gastrointest Endosc* 2006;63(2):204–211.
33. Yanagisawa K, Moriyasu F, Miyahara T, Yuki M, Iijima H. Phagocytosis of ultrasound contrast agent microbubbles by Kupffer cells. *Ultrasound Med Biol* 2007;33(2):318–325.
34. Overman D. Olympus announces exclusive US co-marketing agreement with Bracco diagnostics Inc. *AXIS Imaging News* 2022. https://www.prnewswire.com/news-releases/olympus-announces-exclusive-us-co-marketing-agreement-with-bracco-diagnostics-inc-301550493.html.