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Three-dimensional quantitative ultrasound for detecting lymph node metastases

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ABSTRACT

Purpose: Detection of metastases in lymph nodes (LNs) is critical for cancer management. Conventional histological methods may miss metastatic foci. To date, no practical means of evaluating the entire LN volume exists. The aim of this study was to develop fast, reliable, operator-independent, high-frequency, quantitative ultrasound (QUS) methods for evaluating LNs over their entire volume to effectively detect LN metastases.

Methods: We scanned freshly excised LNs at 26 MHz and digitally acquired echo-signal data over the entire three-dimensional (3D) volume. A total of 146 LNs of colorectal, 26 LNs of gastric, and 118 LNs of breast cancer patients were enrolled. We step-sectioned LNs at 50-μm intervals and later compared them with 13 QUS estimates associated with tissue microstructure. Linear-discriminant analysis classified LNs as metastatic or nonmetastatic, and we computed areas (Az) under receiver-operator characteristic curves to assess classification performance. The QUS estimates and cancer probability values derived from discriminant analysis were depicted in 3D images for comparison with 3D histology.

Results: Of 146 LNs of colorectal cancer patients, 23 were metastatic; Az = 0.952 ± 0.021 (95% confidence interval [CI]: 0.911–0.993); sensitivity = 91.3% (specificity = 87.0%); and sensitivity = 100% (specificity = 67.5%). Of 26 LNs of gastric cancer patients, five were metastatic; Az = 0.962 ± 0.039 (95% CI: 0.807–1.000); sensitivity = 100% (specificity = 95.3%). A total of 17 of 118 LNs of breast cancer patients were metastatic; Az = 0.833 ± 0.047 (95% CI: 0.741–0.926); sensitivity = 88.2% (specificity = 62.5%); sensitivity = 100% (specificity = 50.5%). 3D cancer probability images showed good correlation with 3D histology.

Conclusions: These results suggest that operator- and system-independent QUS methods allow reliable entire-volume LN evaluation for detecting metastases. 3D cancer probability

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images can help pathologists identify metastatic foci that could be missed using conventional methods.

1. Introduction

For many cancers, accurate detection of metastases in lymph nodes (LNs) is crucial to determine the disease stage using the American Joint Committee on Cancer tumor-node-metastases staging system. Changes in the node status affect treatment and management. The latest edition categorizes micrometastases (0.2–2 mm) and isolated tumor cells (<0.2 mm or <1000 tumor cells) separately from macrometastases. Micrometastases are considered to be clinically significant and positive for metastases [1].

For all cancers, pathologists currently perform a microscopic histologic examination of surgically dissected LNs. For colorectal cancer and gastric cancer, only one central histological section of each LN is usually evaluated for metastases, regardless of LN size [2,3]. For invasive carcinoma of the breast, the College of American Pathologists recommends that each LN should be sliced parallel to the long axis of the LN at a spacing of 2 mm. These slices are then submitted for microscopic examination with at least one representative hematoxylin and eosin (H&E)–stained thin section obtained from the surface of each slice examined histologically [4].

To date, no method is clinically available for examining LNs in their entire volume to detect metastases. Molecular studies such as reverse transcription polymerase chain reaction have been reported [5–8], but continue to be a research topic and have not been adopted for clinical practice. There is some consensus in the literature that treatment decisions should not yet be based on these techniques [1–4,9–12]. The reference standard remains histologic examination of H&E histology, and occasionally additional sections from the specimen may be required for subsequent special staining, such as immunohistochemical methods [4,13,14]. With the conventional method, unless the metastases are included in the section examined microscopically, metastases, particularly micrometastases, may be missed [15–22].

Breast sentinel LN biopsy now is well established in the United States for clinically node-negative axillas [9,23]. Touch-prep imprinting and frozen-section procedures for detection of metastases provide limited sensitivity because of sampling limitations [9,24–28]. Multiple-level step sectioning of specimens has been reported to detect more metastases [15–18,20–22]. Different countries and facilities have reported their own protocols for multilevel step-sectioning at different intervals of axillary sentinel LNs of breast cancer patients [4,29–31], but to date, no international consensus on an optimal histopathology procedure exists [31]. The clinical impact on the outcome of detecting occult micrometastases and isolated tumor cells remains controversial [4,17,21,32–35]. Recently, the need to complete a formal axillary LN dissection in patients with a positive sentinel LN biopsy showing macrometastases or micrometastases in fewer than three nodes has been questioned [36] and remains controversial [37]. However, many of these studies do not account for the potential true residual disease prevalence in axillary LNs, because metastases are detected using limited conventional histopathologic procedures. If a new method could be developed to rapidly assess LNs for suspicion of metastases noninvasively over the entire LN volume before histology processing, the new method would resolve the current controversies and would have broad implications for staging a wide range of cancers.

The aim of our study was to develop a fast, reliable, and operator-independent method for entire-volume LN examination to detect and image LN metastases using high-frequency (HF) quantitative ultrasound (QUS) [38,39]. By using HF ultrasound (i.e., >15 MHz) and digitally acquiring and analyzing the ultrasound echo signals, QUS methods can provide estimates of tissue microstructure on a subresolution scale. Unlike B-mode ultrasound images currently used clinically, QUS methods are operator independent and provide a quantitative means of estimating microscopic-scale tissue properties. These attributes, combined with the ability of three-dimensional (3D) ultrasound scans to acquire data from the full LN volume, enable QUS methods to evaluate the entire LN and detect micrometastases as well as macrometastases. Future clinical 3D QUS systems potentially will enable surgeons and pathologists to detect metastatic LNs with high sensitivity.

2. Materials and methods

2.1. *Enrollment*

A total of 160 patients (44 men and 116 women) with histologically proven colorectal, gastric, and breast cancer, who underwent cancer surgery at the Kuakini Medical Center in Honolulu, Hawaii, were randomly and consecutively enrolled in this prospective study. This patient cohort included 71 patients (all women) with breast cancer, 77 patients (38 men and 39 women) with colorectal cancer, and 12 patients with gastric cancer (6 men and 6 women). The median age for each cancer type was: breast, 65 y (range, 42–93 y; mean, 67.4 y; standard deviation [SD], 12.5 y); colorectal, 74 y (range, 40–95 y; mean, 71 y; SD, 13.1 y); and gastric, 81.5 y (range, 52–93 y; mean, 76.3 y; SD, 14.1 y).

Institutional review boards at the University of Hawaii and the Kuakini Medical Center approved the study protocol. We obtained written informed consent from all patients.

2.2. *Materials*

Study materials were LNs harvested from surgical specimens dissected from previously untreated patients with histologically proven colorectal, gastric, or breast cancer; axillary sentinel LNs that underwent an imprint (i.e., “touch-prep”) cytology procedure satisfied this criterion. The study excluded