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ORIGINAL ARTICLE

Validation of Contrast Enhanced Ultrasound Technique to Wire Localization of Sentinel Lymph Node in Patients with Early Breast Cancer

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Abstract Axillary staging is one of the primary steps in management of Breast cancer patients. Current standard methods including blue dye and radicolloid have limitations and disadvantages. In this study, the feasibility of visualization of lymph node pathways and localization of SLN with the help of CEUS was assessed. 50 patients with early breast cancer diagnosis underwent CEUS and wire localization, methylenblue dye, and isotope scan methods for SLN detection. The pathology findings of the wired SLN were compared with those obtained from, methylenblue dye, and isotope scan methods. Lymph node wiring was successfully performed in 48 patients.Radio-isotope technique detected SLN in all 50 patients while blue-dye succeeded in 48. Sensitivity of CEUS to detect SLN compared with radio-isotope and blue dye methods was 96 % and 100 %, respectively. Considering costs and facilities required to perform radio-isotope technique and complications of blue dye we may accept CEUS with the help of micro-bubble contrasts as a viable alternative. However, more studies with larger sample volumes, using various drugs, and including non-selective population are warranted to better clarify feasibility and accuracy of this technique in comparison with current methods.

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Introduction

Evaluation of the axillary lymph nodes involvement is one of the most important steps for axillary staging in breast cancer. Although axillary lymph node dissection (ALND) is considered the gold standard method for staging, due to its frequent morbidities (e.g. lymph edema and seroma) sentinel lymph node biopsy (SLNB) with the help of blue dye or radiocolloid has become the standard of care for early breast cancer patients [1–2]. These methods have similar results to ALND in terms of overall survival, disease free survival, and regional control [3]. Unfortunately, any of these two methods has its own limitations. Radio-colloid method requires nuclear facilities and Gamma-probe device and exposes the medical personnel and patient to the ionizing radiation. On the other hand, blue dye causes a long-lasting tattoo [4] and may result in anaphylactic shock [5]. Thus, some new studies have been conducted to introduce new techniques to find out the sentinel lymph node (SLN) [6]) among which contrast enhance ultrasound (CEUS) is of certain importance and advantages [7–8]. In this study, the feasibility of visualization of lymph node pathways and localization of SLN with the help of CEUS was assessed..

Methods and Patients

This study was approved by the ethics committee of our university of medical sciences. Written informed consents were obtained from the intended participants. All patients were treated from March 2013 to August 2014 in Iranian National Cancer Institute affiliated with ministry of health.

As most of the previous data was from experimental animal studies, we operated the first five patients as a pilot to set up the technique. Data of these five cases were not included in the final analysis.

Fifty females diagnosed with breast cancer less than 3 cm in size and negative lymph nodes on clinical and gray-scale sonographic basis were included in our study. Patients with past medical history of ischemic heart disease, hypertension, or any major co-morbidity were excluded. Also, only patients with localized tumor on digital mammography entered the study and those with multi-focal or multi-centric lesions did not. As the reliability of SLNB after neo-adjuvant treatment is controversial [9], these patients were excluded as well. All patients underwent core needle biopsy (CNB) for pathological diagnosis and none had previous surgical scar in her breasts.

On the day of operation, patients were injected 100 mc of Technetium 99 (Nanocall, GE Healthcare, Chicago, USA) subdermally within the territory of the tumor. Then, they underwent conventional gray scale sonography of axilla and breast. About 0.2–0.3 ml of FDA-approved DEFINITY® (Perflutren Lipid Microsphere) (http://www.definityimaging.com/how-faq.html) was activated and injected according to the instructions (http://www.definityimaging.com/how-administration.html). Without massaging the injection site, within 1–2 min micro-bubbles will pool in the first axillary lymph node, remain there for 5–6 min, and are washed away through efferent lymphatic pathway toward the next lymph nodes. After observing the first lymph node, radiologist will anesthetize the overlying skin with 1 cc of Lidocaine 2 % and a wire (Bard, Dual OK, UK) were inserted into this node or concomitantly enhanced lymph nodes. Then, the patient was delivered to the operation room and after induction of anesthesia, 1 ml of Sulphan blue (Patent Blue Violet; Wako Pure Chemical Industries, Osaka, Japan) was injected subdermally in peri-areolar tissue. The best location for incision was determined according to the Gamma probe. Through a 3–4 cm incision, the wired lymph node was detected which was contrasted with blue dye and Tc^{99m} uptake. If blue dye or Tc99m showed other lymph nodes as SLN, those would be also excised and labeled separately for frozen-section procedure. Meanwhile, either mastectomy or breast conserving surgery was performed and finally, ALND would be performed in case of any positive results from frozen sections.

Results

Average of age was 53 (23–69) years and the mean tumor size was 23 (8–30) mm. Of all the patients, 41 showed invasive ductal carcinoma, 6 patients invasive lobular carcinoma, and the remaining three had medullary carcinoma in their pathological examinations. Tumor location was UOQ in 29, UIQ in 12, LOQ in 7, and LIQ in 2 patients. Tumor was discovered in 11 patients during routine screening examinations and was symptomatic in 39 (palpation of a mass in 28, bloody discharge in 6, and pain in 5) cases.

Lymph node wiring was successfully performed in 48 patients whereas in two patients no bubbles were observed after 15 min passed in spite of two contrast injections. Simultaneous appearance of bubbles in two different lymph nodes was observed in four patients where two wires were inserted in each node. Transit time from injection to pooling of micro-bubbles was between 120 to 290 s where the mean distance between injection site to the axillary lymph node was 14 cm and the depth of lymph nodes from the skin was 35 mm (20–65 mm). Localized lymph nodes per patient were 1.1 on average.

Radio-isotope technique detected SLN in all 50 patients while blue-dye succeeded in 48. In two patients, neither blue-dye nor CEUS could detect SLN where both lymph nodes were 60 mm or more deep from skin. In these two patient there was just one SLN that detected with radicolloid.

In 6 patients, 2 SLNs were detected by using radio-isotopes. Of these patients, 4 were also positive with CEUS and 5 with blue dye methods. Of 56 lymph nodes sent for frozen sections, 9 lymph nodes (of 7 patients) were tumoral, where ALND was performed subsequently. Sensitivity of CEUS to detect SLN compared with radio-isotope and blue dye methods was 96 % and 100 %, respectively..

Discussion

To introduce a substitute method for the current SLNB techniques, we should consider feasibility, cost-effectiveness, resource limitations, and complications. it seems that the method discussed in this paper meet above mentioned requirements.

Micro-bubble contrast agents are a group of drugs which contain gas bubbles covered by a protective layer known as stabilizer. These bubbles are less than 2 μm in size and their covering layer is made of albumin, phospholipids, etc. These drugs are used intra-venously to enhance diagnostic value of ultrasound in cardio-vascular diseases or tumors of various organs (e.g. liver, kidney, eye, etc) [10].

Subcutanous injection of these drugs has been used by some for mapping of tumors' lymphatic drainage with the help of CEUS. In 2004, Goldberg et al. used peri-tumoral injections of these drugs in six swine models of melanoma and succeeded to visualize lymphatic pathways and detected SLN [11]. One year later, the same researchers published their experience with Sonazoid in various animal models and utilization of scanning electron microscopy to describe movement and accumulation of contrast in lymphatic pathways until reaching the SLN [12]. In 2006, Lurie and colleagues administered the drug peri-tumorally in 10 dogs with head and neck cancers and succeeded in detecting SLN in eight of them [13].

The first human study was reported by Omoto et al. in 2009 where Sonazoid was injected in 20 females with breast cancer which resulted in detection of SLN in 14 of them and subsequent excisional biopsy [14].

Sever and colleagues in 2009 and 2011 SLN wire localization under CEUS after Sonovue injection and showed that sensitivity of this procedure in detecting SLN compared with current standards (i.e. radioisotope or blue dye) is about 89 % [15–16]. The same authors published a paper in 2010 to explain details of CEUS method with micro-bubble contrasts and described dynamics of the bubbles and its hardware requirements [17].

As lymphatic pathways may undergo some changes after excisional biopsy [18], in this study, we just included patients whose diagnosis was confirmed by core needle biopsy rather than excisional biopsy. On the other hand, as the chance of lymph node involvement is directly related to the size of primary tumor (i.e. T stage) [19], only patients with T-stage below 3 cm were enrolled. Although SLNB after neo-adjuvant chemotherapy has been showed by some studies to be reliable, it has not received widespread acceptance [9, 20]. So, we decided to exclude patients who had undergone chemotherapy.

In 2010, Sever et al. performed CEUS to localize SLN and found its sensitivity to be 89 % [16] while another study by the same group in 2013 reported the sensitivity to be around 61 % [21]. Considering the learning curve and more experience with this technique, we should expect an improvement in sensitivity during these years which did not happen. This reduction in sensitivity might be explained by different techniques to evaluate the SLN: in their 2010 study, they wired the SLN and extracted the lymph node totally but in the 2013 study, CNB was used to evaluate the SLN. As in some cases the lymph node may not be totally tumoral, needle biopsy may result in false negative results and reduce the sensitivity. Therefore, we used wiring and total node removal in our study.

Omoto and colleagues could extract 1.1 axillary lymph nodes on average from each patient with the help of CEUS using Sonazoid where duration for contrast agent to reach axillary nodes from injection site was 5.3 min (2–20 min) [14]. In Sever's studies which used Sonavue, on average 2 lymph nodes (1–5) were removed with 10–30 s duration [15]. Higher speed of Sonavue's micro-bubbles may mislead the radiologist about the correct SLN as the contrast exits lymph nodes so fast. So, we used Definity in our study whose micro-bubbles according to previous animal studies are faster than Sonazoid and slower than Sonovue [13–14]. Definitiy and Sonovue are both approved by FDA (http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/CardiovascularandRenalDrugsAdvisoryCommittee/UCM252880.pdf) and have nearly similar applications and complications.

In this study, we could detect lymphatic pathways and SLN by peri-tumoral injection of Definity with 96 % accuracy and sensitivity. Higher rates of accuracy and sensitivity in our case series may have several underlying reasons. First, none of the patients in our study had experienced any previous surgery which could disturb anatomical lymphatic pathways. Also, in contrast with previous studies which recommended massaging the injection site [14–17], we did not do it for any of the patients in fear of destroying the micro-bubbles.

Interestingly, in two patients whose SLNs were detecting only with radio-isotope technique, both lymph nodes were located 60 mm deep from skin and it seems that fatness may reduce the sensitivity.

Conclusion

Considering costs and facilities required to perform radioisotope technique and complications of blue dye such as long-lasting tattoos, blue-stained axillary bed, and anaphylaxis, we may accept CEUS with the help of micro-bubble contrasts as a viable alternative. However, more studies with larger sample volumes, using various drugs, and including non-selective population are warranted to better clarify feasibility and accuracy of this technique in comparison with current methods.

Conflict of Interest The authors declare that they have no conflict of interest.

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