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Contrast-Enhanced Sonography for Detection of Secondary Lymph Nodes in a Melanoma Tumor Animal Model

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Abstract

Objectives—To investigate the use of contrast-enhanced ultrasound imaging (US) for detection of secondary lymph nodes (LNs) in a naturally occurring melanoma swine model compared to surgery and pathologic assessment.

Methods—Twenty-seven Sinclair swine were studied. The perfluorobutane microbubble contrast agent Sonazoid (GE Healthcare, Oslo, Norway) was administered (1.0 mL total dose) around the melanoma, and contrast-enhanced US was used to localize contrast-enhanced sentinel lymph nodes (SLNs). Then Sonazoid (dose, 0.25–1.0 mL) was injected into the SLNs to detect contrast-enhanced efferent lymphatic channels and secondary LNs. After peritumoral injection of blue dye, a surgeon (blinded to the contrast-enhanced US results) performed a radical LN dissection. Contrast-enhanced US was used to guide removal of any enhanced secondary LNs left after radical LN dissection. Clustered conditional logistic regression analyzed the benefit of contrast-enhanced US-directed secondary LN dissection over radical LN dissection using pathologic findings as the reference standard.

Results—A total of 268 secondary LNs were resected, with 59 (22%) containing metastases. Contrast-enhanced US detected 92 secondary LNs; 248 were identified by radical LN dissection; and 68 were identified by both methods. Metastases were detected in 20% (51 of 248) and 40% (37 of 92) of the secondary LNs identified by radical LN dissection and contrast-enhanced US, respectively. Thus, secondary LNs detected by contrast-enhanced US were nearly 5 times more likely to contain metastases than secondary LNs removed by radical LN dissection (odds ratio, 4.8; $P < .0001$). Twenty-two of the 180 secondary LNs (12%) identified only by radical LN dissection contained metastases, whereas contrast-enhanced US identified 20 secondary LNs after the surgeon completed the radical LN dissection, of which 8 (40%) contained metastases.

Conclusions—Secondary LNs can be detected by using contrast-enhanced US after injection of Sonazoid into SLNs. Secondary LNs detected with contrast-enhanced US are significantly more likely to contain metastases than those removed by radical LN dissection.

Keywords

cancer; contrast; lymph nodes; sonography

Detection of sentinel lymph nodes (SLNs) followed by histologic assessment is important for the management of patients with a variety of cancers.^{1–3} Currently, two techniques are used to map lymphatic drainage from malignant tumors and localize SLNs: ie, injection of blue dye (to provide visual identification of lymphatic structures) and injection of radiopharmaceuticals followed by evaluation with a gamma camera (ie, lymphoscintigraphy) or intraoperatively with a gamma probe.^{4–8} Once the SLNs are localized, they are then biopsied or surgically excised and histopathologically assessed to stage disease. If the SLN is found to contain metastases, radical lymph node (LN) dissection may be performed in an effort to remove secondary LNs so that they too can be evaluated histologically.^{9–11}

Our group has previously reported on the use of contrast-enhanced ultrasound imaging (US) to detect lymphatic channels (LCs) and SLNs after interstitial injections of microbubble-based US contrast agents (termed “lymphosonography”) in several animal species.^{12,13} These investigations have included the evaluation of contrast-enhanced US for detection and characterization of SLNs in a swine model that has naturally occurring melanoma tumors.^{14,15} The results of those studies concluded that lymphosonography was statistically better than lymphoscintigraphy for detection of SLNs in this melanoma swine model. A few published reports have described the use of contrast-enhanced US for SLN mapping in humans, although currently there are no US contrast agents approved for this application.^{16,17} However, these investigations were performed in a limited number of patients, and none attempted to localize secondary LNs.

Although current methods used for SLN mapping (ie, blue dye–guided surgery and lymphoscintigraphy) have proven reliable for localization of SLNs, there is no imaging technique that is used routinely to localize secondary or “second-echelon” LNs. We hypothesized that by injecting Sonazoid directly into contrast-enhanced US–detected SLNs, it would be possible to identify contrast-enhanced efferent LCs which, in turn, could be traced to secondary LNs. This study was an attempt to determine the capabilities of contrast-enhanced US to detect secondary LNs and compare the results to radical LN dissection and pathologic assessment in a naturally occurring melanoma swine model.

Materials and Methods

Animal Model

A total of 27 Sinclair swine (Sinclair BioResources, Columbia, MO) with melanoma tumors were evaluated in this study. The Sinclair swine model has a heritable trait of naturally occurring melanoma with an incidence in piglets of 54% at birth rising to 85% at 1 year of

age.¹⁸ The melanomas, which can be multiple on a single swine, are similar to human melanomas in their clinical appearance and histopathologic characteristics. Swine that have melanoma tumors have a 70% incidence of metastasis to regional draining SLNs.¹⁹ The swine included in this study ranged in weight from 1.8 to 30.0 kg (average, 11.4 kg).

The studies described herein were performed in an ethical and humane fashion under supervision of a veterinarian and fully conformed to the National Institutes of Health guidelines for the use of laboratory animals.²⁰ All protocols were approved by the Institutional Animal Care and Use Committee.

The swine were premedicated with an intramuscular administration of Telazol (Fort Dodge Animal Health, Fort Dodge, IA) at a dose of 3 to 5 mg/kg and placed on a warming blanket to maintain body temperature. A face mask with 4% to 5% isoflurane (Isothesia; Abbott Laboratories, North Chicago, IL) was used for induction of anesthesia and maintained with 2% to 5% isoflurane during the entire procedure.

Ultrasound Imaging

Conventional US and contrast-enhanced US were performed using an Acuson Sequoia 512 US machine (Siemens Medical Solutions, Mountain View, CA) and a high-frequency, broad-bandwidth linear array transducer (L15-8W). Contrast-enhanced US was performed using Cadence pulse sequencing^{21–23} at a low acoustic power (as indicated by the mechanical index) to minimize contrast agent microbubble destruction. Baseline (pre-contrast administration) US and contrast-enhanced US scans were performed by the same investigator (D.A.M.), with more than 10 years of contrast lymphosonographic experience, and all data were digitally recorded with selected still images and cine loops stored for documentation and subsequent analyses.

Baseline US was performed to measure the melanoma tumor in 3 dimensions. A lipid-stabilized suspension of perfluorobutane microbubbles (Sonazoid; GE Healthcare, Oslo, Norway) with a median diameter of 2.4 to 3.5 μm ²⁴ was then administered in 4 injections of 0.25 mL (1.0 mL total dose) at equidistant locations (3-, 6-, 9-, and 12-o' clock) around the melanoma tumor. The injection sites were massaged for up to 5 minutes to augment lymphatic uptake of Sonazoid. Contrast-enhanced US scanning was then performed around the melanoma in an attempt to identify contrast-enhanced LCs, which were continuously followed with real time contrast-enhanced US to localize contrast-enhanced SLNs. A variety of anatomic areas were assessed according to the location of primary melanoma, the course of draining LCs, and the locations of the SLNs. Knowledge of the swine lymphatic anatomy was used to determine the most likely locations of the enhanced LCs and SLNs. Details about the locations of the LCs and SLNs, including their sizes (in 3 orthogonal dimensions), locations, and anatomic relationships to recognized anatomic landmarks, were recorded to permit correlation with the results of blue dye-guided surgical dissection.

After localization of all contrast-enhanced SLNs, contrast-enhanced US was used to guide insertion of a 22-gauge needle into each contrast-enhanced SLN, and 0.25 to 1.0 mL (based on the size of the node) of Sonazoid was transcutaneously injected into each SLN whenever possible. Contrast-enhanced US was then used to detect the efferent contrast-enhanced LCs

that coursed from the injected SLNs to other LNs, which were considered secondary LNs. The sizes and locations of all contrast-enhanced secondary LNs were recorded for later analysis. Contrast agent injections for both the primary tumors and SLNs were performed by the same investigator (J.B.L.), with more than 10 years of contrast lymphosonographic experience.

Blue Dye–Guided Surgery

After the contrast-enhanced US evaluation was completed, individual 0.25-mL injections of blue dye (1% Lymphazurin; Ben Venue Laboratories, Inc, Bedford, OH) were administered around the melanoma tumor at 12-, 3-, 6-, and 9-o'clock positions (ie, in the same locations as was Sonazoid), and the injection sites were massaged for 5 minutes to facilitate lymphatic uptake of the dye. The swine were then euthanized with an intravenous injection of Euthasol (Delmarva Laboratories, Midlothian, VA) at a dose of 0.25 mL/kg in accordance with the guidelines for the euthanasia of animals by the American Veterinary Medical Association.²⁵

A surgeon (A.C.B.) with expertise in oncology and LN surgery used the blue dye and contrast-enhanced US findings to guide surgical excision of the SLNs and then used the blue dye and professional judgment to perform a radical dissection of the LNs that were considered to represent the melanoma's secondary LNs. During the radical LN dissection, the contrast-enhanced US investigators pointed out the locations of the primary tumors and the contrast-injected SLNs but did not provide any information regarding secondary LNs in terms of numbers or locations. The surgeon was blinded to the findings regarding the locations of the secondary LNs identified with contrast-enhanced US. The anatomic locations of all resected specimens were recorded for comparison to the contrast-enhanced US findings. After the surgeon performed the radical LN dissection, the regions of interest with pre -surgical contrast-enhanced US–identified secondary LN information (including both surgically opened and unopened regions) were thoroughly rescanned using contrast imaging in an attempt to identify any remaining contrast-enhanced secondary LNs in situ missed by the surgeon. If additional contrast-enhanced secondary LNs were detected, they were surgically excised under contrast-enhanced US guidance and processed as described below.

Since the contrast microbubbles can stay in LNs up to 6 hours, enhancement can still be seen within LNs even after removal by radical LN dissection. Thus, all surgically resected LN specimens were immediately assessed ex vivo with contrast-enhanced US to determine the presence or absence of Sonazoid in the LNs and visually by the surgeon to determine whether the specimens were stained with blue dye. The resected specimens were then measured and photographed before being sent for histopathologic assessments.

Histopathologic Assessments

Histopathologic examinations using paraffin sections stained with hematoxylin and eosin were performed on all surgically excised LN specimens to detect or rule out the presence of metastatic tumor deposits. Tumor cells in the LN sections were detected according to established cytologic characteristics of melanoma cells.²⁶ Histopathologic results were read

by an experienced dermatopathologist (A.W.) and used as the reference standard to characterize LNs as benign or malignant.

Statistical Analysis

The number of swine that had secondary LN metastases and the percentage of malignant secondary LNs identified with contrast-enhanced US and removed by radical LN dissection were tabulated. A clustered conditional logistic regression analysis of correlated proportions (equivalent to a clustered McNemar test) was used to calculate the benefit of contrast-enhanced US-directed secondary LN dissection over radical LN dissection using Stata 9.0 software (StataCorp, College Station, TX). Clustered logistic regression was chosen to compensate for the lack of independence in secondary LNs within one animal.

An odds ratio (OR) was calculated for the correct detection of metastases (ie, malignant secondary LNs detected by a technique and benign secondary LNs not detected were counted as positive outcomes). $P < .05$ was considered an indication of statistical significance. Finally, the volume of the primary melanoma (calculated using the formula for a prolated ellipse: ie, $\pi/6 \times \text{length} \times \text{width} \times \text{height}$) and its location were compared to the number of metastatic secondary LNs. The first analysis used unpaired t tests, and the latter used the Wilcoxon rank sum test.

Results

A single melanoma tumor on each of the 27 swine was selected to be imaged as described above. The melanoma tumors studied were located in a variety of anatomic locations, including the head and neck ($n = 1$), dorsal body ($n = 18$), ventral body ($n = 5$), and hind limb ($n = 3$). The mean diameter $\pm$ SD of the melanomas was 34 ± 17 mm (range, 5–85 mm), and the mean volume of the melanomas was $78,452 \pm 136,962$ mm³ (range, 419–717,435 mm³).

Typically, contrast-enhanced LCs could be identified with contrast-enhanced US within seconds after injection of Sonazoid around the melanoma tumor, and movement of contrast within LCs could be readily visualized with real-time contrast-enhanced US. Sentinel LNs and secondary LNs that received Sonazoid-enhanced lymphatic drainage were identified by an increase in echogenicity (ie, contrast enhancement) of the nodal parenchyma. Contrast enhancement of the SLNs ranged from complete and homogeneous to heterogeneous (Figure 1). A total of 80 SLNs were identified with contrast-enhanced US after injection of Sonazoid around the melanoma tumor. The minimum and maximum numbers of SLNs detected in any one swine were 1 and 9, respectively (average, 2.96 SLNs per tumor).

Sonazoid was injected under US guidance into 65 of the 80 SLNs (81.3%) identified with contrast-enhanced US, whereas 15 SLNs could not be successfully injected because they were too small, deeply located, or both. Ultrasound-guided injection of Sonazoid into an enhanced SLN typically resulted in increased enhancement of the SLN (Figure 2). After the SLNs were injected with Sonazoid, efferent LCs could be identified with contrast-enhanced US and then traced down to the next tier of nodes. These enhanced LNs that were detected after Sonazoid injection were considered to represent secondary LNs (Figure 3). The

contrast-enhanced SLNs were often located in the superficial areas (ie, inguinal, lateral flank, and superficial cervical regions), whereas the secondary LNs that originated from these SLNs were often found in deeper areas (ie, mediastinum, deep pelvic, and retroperitoneal regions), which can be up to 35 cm away from the SLN (Figures 4 and 5). In addition, contrast-enhanced US was able to reconfirm the enhancement ex vivo in the resected secondary LNs identified by in vivo contrast-enhanced US (Figure 6).

A total of 268 LNs were surgically resected, with a mean diameter of 9.0 ± 5.7 mm and a mean volume of 1752.7 ± 3361.7 mm³. Ninety-two secondary LNs were identified by contrast-enhanced US, and 248 LNs presumed to represent secondary LNs were removed by radical LN dissection. There was no statistically significant difference between the sizes and volumes of secondary LNs identified by contrast-enhanced US and radical LN dissection (9.6 ± 5.6 versus 8.8 ± 5.6 mm; $P > .21$; and 2198.4 ± 4287.0 versus 1624.4 ± 2829.5 mm³; $P > .07$; Table 1). Sixty-eight secondary LNs were identified by both contrast-enhanced US and the surgeon.

A total of 59 of the 268 resected secondary LNs (22%) contained metastases. Metastases were detected in 20.6% (51 of 248) of the secondary LNs removed by radical LN dissection and in 40.2% (37 of 92) of the secondary LNs identified with contrast-enhanced US (Table 2). There were 22 secondary LNs missed by contrast-enhanced US that were subsequently found to contain metastases, whereas 8 secondary LNs missed by the surgeon were confirmed as metastases. Thus, those secondary LNs that were identified by contrast-enhanced US were nearly 5 times more likely to be positive for metastases compared to secondary LNs removed by radical LN dissection (clustered logistic regression of proportions, OR, 4.8; $P < .0001$).

In 12 swine, a total of 20 contrast-enhanced secondary LNs were found with contrast-enhanced US after the surgeon completed the radical LN dissection. All of these secondary LNs were subsequently resected and histopathologically evaluated, which determined that 40% (8 of 20) contained metastases. Of the 20 radical LN dissection–missed secondary LNs, there were 6 secondary LNs located in the mediastinum, 5 posterior to the thoracic aorta, 3 in the deep pelvis near the iliac vessels, 2 posterior to the aorta/vena cava, 2 posterior to the kidney, and 2 in the deep cervical region (Figures 7 and 8).

There was a statistically significant difference between secondary LNs with and without metastases depending on the melanoma tumor size (ie, maximum diameter, 34.8 ± 16.7 versus 39.8 ± 15.0 mm; $P = .038$). There were also differences in the numbers of secondary LNs that contained metastases related to the primary tumor locations. Tumors on the ventral body and the hind limb were 2 to 3 times more likely to have secondary LNs that contained metastases ($P = .011$) compared to tumors on the head/ neck and dorsal body (Table 3).

Discussion

In current clinical practice, lymphoscintigraphy and blue dye–based mapping are the standard methods for SLN localization in patients with a variety of cancers.^{23,24} The limitations of these techniques include radiation exposure, invasiveness, contrast leakage

beyond the SLN, and the inability to show the internal architecture of the SLNs, which may be important for the mapping and characterization of metastatic nodes. Our group has previously reported on the use of contrast-enhanced US to map LCs and detect SLNs in several animal species.^{12–15} The study of contrast-enhanced US and lymphoscintigraphy for detection of SLNs in a naturally occurring melanoma swine (using blue dye–guided dissection as the reference standard) showed that the accuracy of SLN detection was 81.8% for contrast-enhanced US, which was significantly higher than the 63.2% achieved with lymphoscintigraphy ($P < .0001$).¹⁵ Interestingly, our previous study found that the contrast enhancement of the lymphatic system was confined within the first draining LN (ie, the SLNs) without passing to the next tier of the LN (ie, secondary LNs). This phenomenon is presumably related to the contrast microbubbles being phagocytized by lymphatic tissue.¹⁵

Thus, this project expanded on the concept of using contrast-enhanced US for lymphatic mapping to include localization of secondary LNs by injection of a contrast agent into the SLNs. Our results have confirmed that secondary LNs can be detected with contrast-enhanced US after direct injection of Sonazoid into contrast-enhanced SLNs. Secondary LNs that were identified with contrast-enhanced US were significantly more likely to contain metastases than those removed by radical LN dissection (20.6% versus 40.2%; OR, 4.8; $P < .0001$). It should be pointed out that contrast-enhanced US missed 22 secondary LNs subsequently found to contain metastases, whereas the surgeon missed 8 secondary LNs with tumors. However, radical LN dissection required removal of 248 secondary LNs, whereas lymphoscintigraphy only had to remove 92 secondary LNs to achieve these results. When comparing both methods, radical LN dissection removes regional nodes based on the local anatomy and blue dye mapping, whereas contrast-enhanced US is used to precisely identify true secondary LNs by tracing lymphatic drainage from the injected SLNs. Thus, some radical LN dissection–obtained regional LNs might be beyond the next tier of the lymphatic chain.

In this study, 20 contrast-enhanced secondary LNs in 12 swine were found with contrast-enhanced US after the surgeon completed the radical LN dissection, and 8 (40%) of them contained metastases on pathologic specimens. The reasons for these missed nodes were most likely the locations and distances of the secondary LNs away from the primary tumors and SLNs, such as nodes located in the mediastinum, in the retroperitoneal space, or behind the vessels. Such nodes are not easily accessible and may not be worth the risk of surgical resection unless direct evidence tying them to tumor-involved SLNs exists (as in this study by contrast-enhanced US).

Clinically, identifying and removing all secondary LNs while also limiting the amount of surgery to avoid normal tissue/structure damage and prevent postoperative complications (eg, lymphedema and infection) is a great challenge for surgeons.²⁷ The presence of multiple SLNs is often seen in cancer patients,^{1–3} with approximately 2 to 3 SLNs reported for patients with breast cancer.²⁸ Similarly, a mean of 3 SLNs per tumor (range, 1–9 SLNs) was seen in this animal study; thus, complete evaluation of secondary LNs requires injection of all SLNs that are detected. However, in this study, we could only inject 65 of the 80 contrast-enhanced US–detected SLNs, since some SLNs were too small or deeply located to permit successful injections. Thus, some “true” secondary LNs could be missed by contrast-

enhanced US because of this technique limitation. Another potential limitation of this method is that an incomplete injection (eg, in which contrast microbubbles do not fill the entire SLN) could affect the drainage of microbubbles from the SLN into some secondary LNs. These limitations could possibly be overcome if this technique were applied during open surgery, since the SLNs would be adequately exposed for injection, and secondary LNs would be more accessible for removal.

As might be expected, larger melanomas (based on maximum diameter) were more likely to produce secondary LN metastases ($P = .038$). This study also demonstrated that the number of secondary LNs containing metastases was related to the primary tumor location. Tumors on the ventral body and the hind limb were 2 to 3 times more likely to have secondary LNs with metastases ($P = .011$) compared to tumors of the head/neck and dorsal body. It should be noted that there were a limited number of tumors from the ventral body ($n = 5$) and head and neck ($n = 1$) in this study. Thus, further research with larger numbers will be needed to confirm this observation.

Surgical treatment of melanoma or breast cancer often requires a radical LN dissection procedure. Accurate identification and localization of tumor-draining LNs is critical for nodal staging and to improve the treatment outcome. Lymphatic drainage patterns can be very complex, with multiple LCs leading to different locations and LNs.²⁹ Currently, SLN identification with blue dye injection and lymphoscintigraphy has been extensively used for nodal staging and resection guidance. However, there are no methods available for identification of a true secondary LN before surgery or during radical LN dissection. Instead, surgeons attempt to remove regional LNs based on local anatomy. This process can result in missing secondary LNs with metastatic involvement or requiring extensive exposure of the normal tissue and potential injury of lymphatic structures during radical dissection procedures.^{26,30–32}

Lymphedema is one of the most common complications of breast cancer surgery and radiotherapy, resulting in a poor quality of life. Approximately 15% to 46% of patients have this complication after breast cancer treatment.³³ The risk factors of lymphedema include treatment-related (type of surgery, radiotherapy, and chemotherapy), disease-related (tumor stage, number of LNs excised, and location of the tumor) and patient-related (body mass index, infection, and limb overuse) factors.^{32,34–36} Providing presurgical tumor staging of the LNs and optimizing radical LN dissection surgery are important for diminishing these complications. Although blue dye injection and lymphoscintigraphy are considered by many to be complementary techniques for SLN identification, they are not suitable for localizing true secondary LNs to guide radical LN dissection. By using contrast-enhanced US-guided LN detection, the tumor's SLNs can be identified and continuously traced to the next tier of nodes (ie, the true secondary LNs) for either guiding biopsy or surgical resection.

In conclusion, this study has demonstrated that secondary LNs can be detected with contrast-enhanced US after direct injection of Sonazoid into contrast-enhanced SLNs. Secondary LNs that were identified with contrast-enhanced US were significantly more likely to contain metastases than those removed by radical LN dissection. The potential clinical benefit of this study is the development of a minimally invasive diagnostic imaging

method that can be used to identify lymphatic drainage from tumors to SLNs and onward to secondary LNs. This technique could potentially be performed preoperatively as well as intraoperatively. This innovative imaging method has the potential to fill a void that currently exists in clinical medicine in terms of the ability to identify true secondary LNs in patients with malignancies while obviating or substantially reducing the need to perform radical LN dissections, thus potentially reducing surgical complications.

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Abbreviations

LC	lymphatic channel
LN	lymph node
OR	odds ratio
SLN	sentinel lymph node
US	ultrasound imaging

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Figure 1.
Contrast-enhanced SLN located within the suprapubic area with a measurement of $2.3 \times 2.0 \times 1.1$ cm.

Figure 2.

Ultrasound-guided injection of Sonazoid into an enhanced SLN. **A**, Initial injection of Sonazoid (arrows) into the SLN. **B** and **C**, With continuous injection, extension of enhancement was shown within the SLN. **D**, The contrast agent filled the entire SLN after completion of the injection. Note that contrast-induced shadowing (S) was shown posterior to the node.

Figure 3. Contrast-enhanced US showing 2 secondary LNs (2NA and 2NB) at the iliac region after contrast agent injection into an SLN. UB indicates urinary bladder.

Figure 4. Efferent LCs (arrows) from an SLN (not shown) were imaged and traced to 2 secondary LNs (2LN) in the retroperitoneal region.

Figure 5. Contrast-enhanced US image showing an SLN located in the superficial inguinal area and its secondary LN (2LN) sited at the deep iliac region. UB indicates urinary bladder.

Figure 6. Contrast-enhanced US was used ex vivo to image a resected secondary LN (arrows) to confirm the presence of Sonazoid.

Figure 7.

Dual grayscale (right) and contrast (left) images showing a deeply located contrast-enhanced secondary LN (arrow) in the pelvis after contrast agent injection into the SLN. Note that conventional grayscale imaging alone could not specifically confirm the presence of the node. UB indicates urinary bladder.

Figure 8.

Secondary LN missed by radical LN dissection. **A**, After the surgeon completed the radical LN dissection procedure, an enhanced secondary LN was detected between the aorta (Ao) and left kidney (LK). **B**, This secondary LN with blue dye staining was resected under contrast-enhanced US guidance.

Table 1

Characteristics of Secondary LNs Identified With Contrast-Enhanced US and Radical LN Dissection

	Secondary LNs, n	Node Diameter, mm	Node Volume, mm ³
Contrast-enhanced US	92	9.6 ± 5.6	2198.4 ± 4287.0
Radical LN dissection	248	8.8 ± 5.6	1624.4 ± 2829.5
Total	268	9.0 ± 5.7	1752.7 ± 3361.7
<i>P</i>		>.21	>.07

Data are presented as mean ± SD where applicable.

Table 2

Secondary LN Metastases Identified With Contrast-Enhanced US and Radical LN Dissection

	Positive for Metastases, n (%)	Negative for Metastases, n (%)
Contrast-enhanced US	37 (40.2)	55 (59.8)
Radical LN dissection	51 (20.6)	197 (79.4)
Total	59 (22)	209 (78)

Table 3

Locations of Primary Tumors Related to Secondary LN Metastases

Location of Primary Tumor	Positive for Metastases	Negative for Metastases
Dorsal body (n = 18)	30	137
Ventral body (n = 5)	17	28
Hind limb (n = 3)	11	21
Head and neck (n = 1)	1	7

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