RESEARCH

Quantitative evaluation of vascularity within cervical lymph nodes using Doppler ultrasound in patients with oral cancer: relation to lymph node size

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Objectives: The aim of this study was to quantitatively evaluate the relationship between vascularity within lymph nodes and lymph node size on Doppler ultrasound images of patients with oral cancer.

Methods: A total of 310 lymph nodes (86 metastatic, 224 benign) from 63 patients with oral cancer were classified into 4 groups according to their short axis diameters: Group 1, short axis diameters of 4–5 mm; Group 2, 6–7 mm; Group 3, 8–9 mm; and Group 4, ≥ 10 mm. Vascular and scattering indices of lymph nodes on Doppler ultrasound images were analysed quantitatively. The vascular index was defined as the ratio of blood flow area to the whole lymph node area and the scattering index was defined as the number of isolated blood flow signal units.

Results: For metastatic lymph nodes, the vascular index was highest in Group 1 and decreased as lymph node size increased. The vascular index of benign lymph nodes did not differ significantly among the four groups. The vascular index of metastatic lymph nodes was significantly higher than that of benign lymph nodes in Group 1. For metastatic lymph nodes, the scattering index increased as lymph node size increased and was significantly higher than that of benign lymph nodes in Groups 2–4.

Conclusions: An increase in vascularity is a characteristic of Doppler ultrasound findings in small metastatic lymph nodes. As the metastatic lymph node size increases, blood flow signals become scattered, and the scattering index increases.

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Introduction

A number of reports have shown that ultrasonography is useful for identifying metastatic cervical lymph nodes in oral cancer patients.^{1–4} The diagnostic criteria for differentiating between metastatic and benign lymph nodes are based on size, shape and definition of margins, as well as the presence of hilar or internal echoes. Furthermore, the assessments of vascularity and blood flow distribution within lymph nodes on Doppler ultrasound images have been reported to provide useful diagnostic information.^{5–10} It was reported that metastatic lymph nodes usually show peripheral flow signals

along the periphery of the lymph nodes and/or scattered flow signals in the parenchyma of the lymph nodes,^{5–7} whereas benign lymph nodes show hilar signals branching radially from the hilum in the parenchyma of the lymph nodes.^{8–10}

However, these reports were analysed qualitatively and did not evaluate the relationship between Doppler ultrasound findings and lymph node size. Herman et al¹¹ reported that metastatic lymph nodes histopathologically exhibit definite hypervascularity of the medullary cords, mostly adjacent to the tumour deposit. Furukawa et al¹² stated that blood flow through the hilum of a lymph node distributes around the metastatic focus, resulting in increased blood flow. It has also been speculated that vascularity within metastatic lymph nodes decreases because of augmentation of necrotic areas as the tumour grows in large metastatic lymph nodes.

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Therefore, we speculate that Doppler ultrasound findings of metastatic lymph nodes depend on lymph node size. In this study, we quantitatively analysed the relationship between vascularity within lymph nodes on Doppler ultrasound images and lymph node size. The difference in Doppler ultrasound findings between metastatic and benign lymph nodes was evaluated for each lymph node size.

Materials and methods

Patient population

63 patients with oral squamous cell carcinoma (SCC) who had undergone pre-operative neck Doppler ultrasound and neck dissection at our hospital between April 2003 and May 2008 were enrolled in this study. There were 37 men and 26 women, ranging in age from 23 to 91 years (median 68 years). The primary tumour was in the tongue in 19 patients, in the lower gingiva in 21, in the upper gingiva in 6, in the buccal mucosa in 8, in the floor of the mouth in 8 and in the submandibular gland in 1 patient. The time between neck Doppler ultrasound and neck dissection was 1–25 days (median 10 days).

In total, 530 cervical lymph nodes were detected with Doppler ultrasound and diagnosed histopathologically. We excluded small lymph nodes with a short axis diameter of ≤ 3 mm on ultrasound images, owing to the difficulty in distinguishing between signal noise and blood flow signals on Doppler ultrasound images. A total of 220 lymph nodes were excluded and 310 lymph nodes (86 metastatic, 224 benign) were evaluated in this study.

These 310 lymph nodes were classified into 4 groups according to their short axis diameters as measured on ultrasound images (Table 1): Group 1, short axis diameters of 4–5 mm; Group 2, 6–7 mm; Group 3, 8–9 mm; and Group 4, ≥ 10 mm (median diameter, 12 mm; range, 10–27 mm).

Informed consent was obtained from all patients who participated in this clinical investigation.

Doppler ultrasound acquisition

Power Doppler ultrasound was performed with a Sequoia 512 (Acuson, Mountain View, CA). Cervical lymph nodes were imaged using a wide bandwidth linear transducer (15L8w). The aperture of the transducer had a long length of 6 cm and a short length of 0.5 cm. The common settings in B-mode were a central frequency of 12 MHz and a gain of 80 dB. The settings in power

Doppler mode were a central frequency of 10 MHz, a velocity scale of 1.3 cm s^{-1} , and a CD gain of 40 dB. The probe was located parallel to the inferior border of the mandible at neck level I and perpendicular to the long axis of the neck at neck levels II–V. Ultrasound examinations were performed by three radiologists (TK, KM and KY), who determined the maximum length of the maximum cut surface of the lymph node to be the long axis and the longest dimension perpendicular to the long axis to be the short axis diameter on the monitor of the ultrasound equipment.

Doppler ultrasound image evaluation

All data were acquired in digital imaging and communications in medicine (DICOM) and transferred to the server of a picture-archiving and communication system (PACS) (Centricity PACS SE-J; GE Healthcare, Milwaukee, WI). The data were converted from DICOM to Joint Photographic Experts Group (JPEG) format and transferred to a personal computer (VGN-Z; Sony, Tokyo, Japan) using a CD rom. We analysed the JPEG images with application software (Adobe Photoshop CS3, Adobe Systems, San Jose, CA).

Lymph node images of the maximum cut surface were analysed with two indices, a vascular index and a scattering index. The vascular index indicates the abundance of blood flow on a Doppler ultrasound image. The scattering index indicates the degree of vessel discontinuity presented on a Doppler ultrasound image. The methods for measuring these two indices are as follows (Figure 1):

- (1) The boundary line between the lymph node and the background was drawn free-hand and the number of pixels in the extracted whole lymph node was counted.
- (2) Flow signals within the lymph node were chosen by colour assignment and the number of pixels for the chosen flow signals was counted. The input value for colour assignment was 150.
- (3) The vascular index was defined as the number of pixels in the flow signals divided by the number of pixels in the whole lymph node, presented as a percentage. The number of isolated flow signal units in the lymph node parenchyma was counted. Isolated flow signal units of ≤ 3 pixels were excluded as noise signals.
- (4) The scattering index was defined as the number of isolated flow signal units in the lymph node parenchyma.

Table 1 Characteristics of lymph nodes evaluated in this study

Group	Short axis diameter (mm)	No. of lymph nodes (%)		
		Benign	Metastatic	Total
Group 1	4–5	160 (91.4)	15 (8.6)	175 (100)
Group 2	6–7	39 (66.1)	20 (33.9)	59 (100)
Group 3	8–9	16 (48.5)	17 (51.5)	33 (100)
Group 4	≥ 10	9 (20.9)	34 (79.1)	43 (100)

Figure 1 Method of measuring vascular and scattering indices. The boundary line between the lymph node and the background was drawn free-hand and the number of pixels in the extracted whole lymph node was counted. Flow signals within the lymph node were chosen by colour assignment and the number of pixels for the chosen flow signals was counted. The input value for colour assignment was 150. The vascular index was defined as the number of pixels in the flow signals divided by the number of pixels in the whole lymph node, presented as a percentage. The number of isolated flow signal units in the lymph node parenchyma was counted. Isolated flow signal units of ≤ 3 pixels were excluded as noise signals. The scattering index was defined as the number of isolated flow signal units in the lymph node parenchyma

Statistical analysis

Statistical analysis was performed with SPSS software for Windows (version 11.01; SPSS, Chicago, IL). Statistical significance was assessed using the Mann-Whitney *U*-test and Games-Howell test. Values of $P < 0.05$ were considered to indicate statistical significance. Receiver operating characteristic (ROC) curve analysis was performed using Analyse-it Method Evaluation Edition software (Analyse-it Software, Ltd., Leeds, UK).

Results

Vascular index

Figure 2 shows the vascular indices for Groups 1–4. For metastatic lymph nodes, the vascular index decreased as lymph node size increased. Group 1 had the highest median vascular index (45.7%) among the four groups. The vascular index differed significantly (Games-Howell test) between Group 1 and Groups 3 ($P = 0.02$) and 4 ($P < 0.01$), and between Groups 2 and 4 ($P = 0.02$). The vascular index of benign lymph nodes did not differ significantly among the four groups.

The median vascular index of benign lymph nodes in Group 1 was 15.7% which differed significantly from the vascular index of metastatic lymph nodes in Group 1 ($P < 0.01$, Mann-Whitney *U*-test; Figures 3 and 4). The vascular index did not differ significantly between metastatic and benign lymph nodes in any other group

(Groups 2, 3 and 4: $P = 0.18$, 0.66 and 0.40, respectively; Mann-Whitney *U*-test).

Given the significant difference in vascular index between metastatic and benign lymph nodes in Group 1, a ROC curve analysis was performed yielding an area under the curve of 0.72 for these lymph nodes which have a short axis diameter of 4–5 mm (Figure 5). Using a vascular index cut-off of 30.6%, the sensitivity and specificity for detecting metastatic lymph nodes were 70.0% and 70.0%, respectively, in Group 1.

Scattering index

Figure 6 shows the scattering indices for Groups 1–4. For metastatic lymph nodes, the median scattering index was 4.0 in Group 1; 9.5 in Group 2; 9.0 in Group 3; and 17.0 in Group 4. The scattering index increased with increasing lymph node size and differed significantly between Group 4 and the other groups (vs Groups 1, 2 and 3: $P < 0.001$, $P = 0.01$ and $P = 0.05$, respectively; Games-Howell test) and between Group 3 and Group 1 ($P = 0.003$; Games-Howell test).

For benign lymph nodes, the median scattering index was 2.0 in Group 1; 4.0 in Group 2; 3.5 in Group 3; and 6.0 in Group 4. The scattering index tended to be higher in Group 4 than in the other groups (vs Groups 1, 2 and 3: $P = 0.242$; $P = 0.279$ and $P = 0.635$; Games-Howell test), but the differences among the groups were not significant.

The scattering index differed significantly between metastatic and benign lymph nodes in Groups 2–4 ($P =$

Figure 2 Vascular indices for benign and metastatic lymph nodes in Groups 1–4. For metastatic lymph nodes, the vascular index was highest in Group 1 and decreased significantly as lymph node size increased. The vascular index of metastatic lymph nodes was significantly higher than that of benign lymph nodes in Group 1

0.01, $P < 0.01$ and $P = 0.03$; Mann-Whitney U -test) (Figures 7 and 8). ROC curve analysis yielded an area under the curve of 0.75 in Group 2; 0.81 in Group 3; and 0.73 in Group 4 (Figure 9). Using a scattering index cut-off of 6 in Group 2, the sensitivity and specificity for detecting metastatic lymph nodes were 79.5% and 75.0%, respectively. Similarly, with a scattering index cut-off of 8 in Group 3, the sensitivity and specificity were 75.0% and 76.5%, respectively, and with a cut-off of 16 in Group 4, the sensitivity and specificity were 77.8% and 66.7%, respectively.

Discussion

We suggest that the vascular index indicates the degree of vascularity within a lymph node. The vascular index of metastatic lymph nodes was highest in Group 1 and decreased as the lymph node size increased. In contrast, the vascular index of benign lymph nodes did not change with changes in lymph node size. Furthermore, the vascular index of metastatic lymph nodes was significantly higher than that of benign lymph nodes in Group 1, and this tended to also be the case in Group 2 ($P = 0.18$).

Figure 3 Metastatic lymph node from a 69-year-old man with squamous cell carcinoma of the right cheek. The lymph node short axis diameter measures 4 mm (Group 1), and the vascular index is 62.1%

Figure 4 Benign lymph node from a 78-year-old man with squamous cell carcinoma of the right tongue. The lymph node short axis diameter is 5 mm (Group 1), and the vascular index is 10.3%

Figure 5 Receiver operating characteristic curves for the detection of metastatic lymph nodes among lymph nodes with a short axis diameter of 4–5 mm (Group 1), using the vascular index. The area under the curve was 0.72. Using a vascular index cut-off of 30.6%, the sensitivity and specificity for detecting metastatic lymph nodes were 70.0% and 70.0%, respectively. (TPF: true-positive fraction. TPF is equal to sensitivity. FPF: false-positive fraction. FPF is equal to 1-specificity.)

Hypervascularity within metastatic lymph nodes during the early stages of metastasis has been shown histopathologically.^{11,13} In agreement with these previous reports, our results reflect increased blood flow in metastatic lymph nodes with angiogenesis during the early stages of metastasis. However, the vascular index of metastatic lymph nodes tended to be lower than that of benign lymph nodes in Group 4, which comprised

the largest lymph nodes. This may be a result of the increased number of blood vessels associated with angiogenesis in the early stages of metastasis becoming displaced by invading tumour cells as the lymph node lesion grows.^{12–15}

The diagnostic accuracy of the vascular index for differentiating between benign and metastatic lymph nodes was analysed. In Group 1, in which the vascular index differed significantly between benign and malignant lymph nodes, the sensitivity and specificity of the vascular index for detecting metastatic lymph nodes were 70.0% and 70.0%, respectively, using a cut-off value of 30.6%. Our values are not high compared with those reported in previous studies investigating the diagnostic accuracy of Doppler ultrasound for detecting metastatic lymph nodes; the sensitivities and specificities in these previous reports were 69–96% and 90–98%, respectively.^{6,10,14–16} However, the high accuracy found in these previous studies may be attributable to their evaluation of lymph nodes of various sizes. As our study was limited to smaller lymph nodes, the achievable accuracy may be lower. We consider an increase in the vascular index to be a useful Doppler ultrasound finding for detecting metastasis in small lymph nodes.

The scattering index, which is the number of isolated flow signal units in the parenchyma of a lymph node, indicates the degree of scattering or discontinuity of flow signals within the lymph node. In the present study, the scattering index increased as the lymph node size increased in metastatic lymph nodes, whereas it did not change for benign lymph nodes, except in Group 4. Furthermore, the scattering index of metastatic lymph nodes was significantly higher than that of benign

Figure 6 Scattering indices for benign and metastatic lymph nodes in Groups 1–4. For metastatic lymph nodes, the scattering index was highest in Group 4 and increased as lymph node size increased. There were no significant differences in the scattering index of benign lymph nodes among the four groups. The scattering index was significantly different between metastatic and benign lymph nodes in Groups 2–4 ($P = 0.01$, $P < 0.01$ and $P = 0.03$, respectively, Mann-Whitney *U*-test)

Figure 7 Metastatic lymph node from an 80-year-old man with squamous cell carcinoma of the left tongue. The lymph node short axis diameter is 8 mm (Group 3), and the scattering index is 16

lymph nodes in Groups 2–4. Thus, scattered blood flow signals were a characteristic of Doppler ultrasound findings for metastatic lymph nodes with a short axis diameter of ≥ 6 mm. Flow signal units are more scattered in larger metastatic lymph nodes because the increased number of blood vessels produced by angiogenesis are displaced by the growth of tumour cells, forcing the blood vessels to move around or through tumour cells. A Doppler ultrasound image shows only one cut surface of an entire lymph node, and thus meandering blood vessels appear as scattered flow signal units. With tumour cell growth, the meandering of blood vessels increases. Therefore, the scattering index, representing the discontinuity of flow signals on Doppler ultrasound images, increases as lymph node size increases.

We analysed the diagnostic accuracy of the scattering index for differentiating between metastatic and benign

Figure 8 Benign lymph node from a 65-year-old man with squamous cell carcinoma of the right oral floor. The lymph node short axis diameter is 8 mm (Group 3), and the scattering index is 2

Figure 9 Receiver operating characteristic curves for the detection of metastatic lymph nodes in Groups 2–4, using the scattering index. Area under the curve was 0.75 in Group 2, 0.81 in Group 3 and 0.73 in Group 4. Sensitivity and specificity, respectively, were: 79.5% and 75.0% in Group 2, with a cut-off value of 6; 75.0% and 76.5% in Group 3, with a cut-off value of 8; and 77.8% and 66.7% in Group 4, with a cut-off value of 16. TPF, true-positive fraction; FPF, false-positive fraction

lymph nodes. With cut-off values of 6, 8 and 16 in Groups 2, 3 and 4, respectively, the sensitivity and specificity for detecting metastatic lymph nodes were, respectively, 79.5% and 75.0% in Group 2; 75.0% and 76.5% in Group 3; and 77.8% and 66.7% in Group 4. These values are not high compared with those of other reports that evaluated the diagnostic accuracy of Doppler ultrasonography for detecting metastatic lymph nodes, as described above for the vascular index. Our results suggest that the scattering index alone provides sufficient diagnostic accuracy for detecting metastasis, regardless of the presence of peripheral flow signals in the lymph node.^{5–7}

The scattering index of benign lymph nodes was higher in Group 4 than in the other three groups, although the difference was not significant, and the specificity was lower in Group 4 than in the other groups. Benign lymph nodes usually show hilar signals branching radially from the hilus in the parenchyma of lymph nodes.^{8–10} However, inflammation can cause an increase in the number of blood vessels in large benign lymph nodes, as found in Group 4, resulting in a discontinuity of flow signals that mimics the signals in metastatic lymph nodes.

We suggest that an increase in the scattering index is a characteristic Doppler ultrasound finding of metastatic lymph nodes with a short axis diameter of ≥ 6 mm.

In conclusion, increased blood flow in lymph node parenchyma is a characteristic Doppler ultrasound finding in small lymph nodes during the early stages of metastasis. Metastasis should be suspected in lymph nodes with a vascular index of 30.6% or greater. An

increase in the number of isolated flow signal units in lymph node parenchyma is also characteristic of medium-to-large metastatic lymph nodes. Our results quantitatively support this characteristic finding, which has been only qualitatively described in previous reports.^{5–10,12–19}

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