Ultrasonography-Guided Core Biopsy of Supraclavicular Lymph Nodes for Diagnosis of Metastasis and Identification of Epidermal Growth Factor Receptor (EGFR) Mutation in Advanced Lung Cancer

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Abstract: The aim of this study was to evaluate the diagnostic performance of ultrasonography (US)-guided core biopsy of a supraclavicular lymph node (SCN) for detecting metastasis and epidermal growth factor receptor (EGFR) mutations.

We included 229 patients who underwent US-guided core biopsy of SCN with lung cancer from January 2011 to December 2013. We evaluated the morphologic characteristics and measured the sizes of SCNs on US and chest computed tomography (CT). The clinical stage, maximum standardized uptake value (SUVmax) on 18F-fluorodeoxyglucose positron emission tomography, and the morphology on US and CT in the positive metastasis were compared with those in the negative metastasis. The prevalence of EGFR mutations of the adenocarcinoma and procedure-related complication was investigated.

The accuracy of US-guided core biopsy of SCN diagnosing metastasis was 97.8% (224/229). The cutoff values (sensitivity; specificity; area under the receiver operating characteristic curve, 95% confidence interval [CI]) of the short-axis dimension of SCN on CT were 0.85 cm (72.3%; 80.6%; 0.808, 95% CI: 0.740–0.875), on US 0.75 cm (73.5%; 84.8%; 0.843, 95% CI: 0.788–0.897), and that of SUVmax 4.05 (79.1%; 81.8%; 0.853, 95% CI: 0.780–0.925). The mutations were positive in 84.8%; 0.843, 95% CI: 0.788–0.897), and that of SUVmax 4.05 (79.1%; 81.8%; 0.853, 95% CI: 0.780–0.925). The mutations were positive in 35.8% with adenocarcinoma. There were no procedure-related complications of US-guided SCN core biopsy.

US-guided SCN core biopsy is a reliable and safe method for detecting metastasis, histologic subtyping, and identifying the EGFR mutation in the advanced lung cancers. It may be a substitute for more invasive lung biopsy as an initial tissue confirmation in the advanced disease.

(INTRODUCTION)

Ultrasonography (US)-guided core biopsy of the supraclavicular lymph node (SCN) is a minimally invasive and cost-effective method that offers fast histological subtyping of patient suspected of having advanced or inoperable lung cancer. To date, cytology using fine needle aspiration of SCN is well known, but its diagnostic yield is unsuitable at approximately 70% according to the previous studies.1–3 We believe that US-guided core biopsy of the SCN can provide a more adequate and conclusive pathological sample than aspiration cytology.

US-guided core biopsy may substitute for other invasive biopsy methods, such as core needle biopsy of the lung cancer, bronchoscopy-guided lung or lymph node biopsy, or video-assisted thoracoscopic surgery, in patients with a palliative setting at their initial presentations. However, little information is available on the diagnostic efficacy of US-guided SCN core biopsy and morphological and size criteria for target SCNs in imaging studies in lung cancer patients.

Positive immunohistochemistry result for thyroid transcription factor 1 is sufficient to diagnose the SCN metastases as being from the primary lung cancer rather than from another primary malignant site.4,5 Epidermal growth factor receptor (EGFR) mutation analysis may also be sufficient. It is important to identify EGFR mutation status in order to predict a better response to first-line EGFR-tyrosine kinase inhibitor therapy in cases of advanced adenocarcinoma.6–9

In our single tertiary referral hospital, many patients with advanced lung cancer have undergone US-guided core biopsy of the SCN in the last 2 years. The present study was conducted to evaluate the diagnostic performance of US-guided core biopsy of the SCN and determine optimal imaging criteria for target SCNs for biopsy for detecting metastasis and EGFR mutations.

MATERIALS AND METHODS

Patient Characteristics

This retrospective study was approved by the Institutional Review Board. The patients’ informed consent was waived. From January 2011 to December 2013, for 2 full years, we identified 333 adult patients (>18 years old) who consecutively underwent US-guided core biopsy for the SCN, which has a single separate code on the electronic medical record.

A cancer center of our hospital has established programs to optimize a multidisciplinary approach by radiologists, pulmonologists, oncologists, radiation oncologists, and thoracic surgeons...
with direct patient contact in our outpatient clinic. According to the consistent approach by 3 multidisciplinary teams for the diagnosis of lung cancer, all patients with enlarged SCN on various imaging modality, with palpable SCN on physical examination, or with maximum standardized uptake value (SUV_{max}) $\geq$2.5 on 18F-fluorodeoxyglucose positron emission tomography (FDG PET) routinely underwent US and US-guided core biopsy during the study period. Among these patients, we excluded 104 patients who fell under the exclusion criteria for the reasons shown in Figure 1.

Thus, a total of 229 patients (151 men and 78 women, aged 36–86 years) who had histologically confirmed lung cancer from the primary site and/or elsewhere including supraclavicular and mediastinal lymph node according to the WHO classification$^{10,11}$ and who were available for all 3 imaging modalities, computed tomography (CT), FDG PET, and the US, were included in this study. For the determination of positive metastasis, the gold standard was the any positive result in any of those modalities AND confirmed in final pathology OR increased size of lymph nodes at 6-month interval follow-up CT with initially negative pathologic results. True-negative metastasis on the US-guided core biopsy was defined as no significant interval change in the SCN on the subsequent CT $\geq$6 months later. Among the 44 patients with negative metastases, 6 patients were excluded because they were unavailable for the subsequent 6-month follow-up CT, and 5 patients with initial false-negative results on core biopsy were included as the positive metastasis group.

Finally, 229 patients who met the eligibility criteria of the positive metastasis of 196 patients (126 men, 70 women, aged 62.4 $\pm$ 11.9) and negative metastasis in 33 patients (25 men, 8 women, aged 64.6 $\pm$ 11.1) were included (Table 1).

The age, sex, smoking history, histologic subtype of the lung cancer, and clinical TNM stage were reviewed. In 147 among 229 patients, the histologic diagnoses were also obtained through a percutaneous core needle (n = 44) and/or bronchoscopy-guided lung biopsy (n = 34) for the intrathoracic lung cancer and in the metastatic lesions (n = 69).

CT Scanning Protocol

For the chest CT examination, 16- or 64-detectors, either SOMATOM Sensation 16 (Siemens Medical Solutions, Forchheim, Germany [180 scans]) or Lightspeed VCT (General Electric Medical Systems, Milwaukee, WI [44 scans]) was used with nonionic contrast enhancement. The scan parameters were 100 effective mA and 120 kV with dose modulation in 16-detector row scanner. The reconstruction intervals were 3- and 5-mm thickness without gaps using the B50 algorithm, and 1-mm thickness with 5-mm gaps using the B60 algorithm. In 64-detector row scanner, the scan parameters were 100–400 mA and 120 kV with dose modulation. The reconstruction intervals were 2.5- and 5-mm thickness without gaps using the lung algorithm, and 1.25-mm thickness with 5-mm gaps using the bone algorithm. All the images were viewed at the mediastinal (width, 450 HU; level, 50 HU) and lung window (width, 1500 HU; level, –700 HU) settings of the axial and coronal images on the picture archiving and communication system.

US Scanning and Biopsy Protocol

The US examinations were performed using either EUB-7500 unit (Hitachi Medical Systems, Tokyo, Japan) or iU22 unit (Phillips Healthcare, Bothell, WA) equipped with a linear high-frequency probe (5–14 MHz). An experienced radiologist (JHB, 20 years’ experience in radiology; thyroid and head and neck tumor specialist) performed all the US-guided core biopsy. After local anesthesia with 1% lidocaine, the US-guided core biopsy was performed using a disposable 18-gauge double-action spring-activated needle (TSK Ace-cut; Create Medic, Yokohama, Japan). Core specimens were obtained 1 or 2 pieces per patient (mean 1.3 pieces, 1.1- or 1.6-cm length excursion).

Using a freehand technique, the end of the biopsy needle was advanced into the node, and then the stylet and the cutting cannula of the needle were sequentially fired. We observed each patient after the biopsy.

Imaging Analysis of the CT, US, and FDG PET

Two independent radiologists (MYK, 18 years’ experience in thoracic radiology; and JC, 2 years’ experience in thoracic radiology) were blinded to the clinical data except that the patient had lung cancer (229 CT, US, and FDG PET scans, respectively). The target SCN had the largest, most representative, and malignant-looking morphology.

Two independent radiologists (MYK, 18 years’ experience in thoracic radiology; and JC, 2 years’ experience in thoracic radiology) retrospectively measured the size of the target SCNs
TABLE 1. Comparison of the Clinical Characteristics of the Patients With Positive Metastasis of the Supraclavicular Lymph Node With Those of the Patients With Negative Metastasis

| SCN Metastasis | Total | Positive (n = 196, %) | Negative (n = 33, %) | P |
|---------------|-------|-----------------------|----------------------|---|
| Age           | Mean ± SD | 62.8 ± 11.8          | 62.4 ± 11.9          | 64.6 ± 11.0 | 0.361 ^ |
| Sex           | Female | 78 (34.1)             | 70 (35.7)            | 8 (24.2)   | 0.198 | |
|              | Male   | 151 (65.9)            | 126 (64.3)           | 25 (75.8)  | 0.208 | |
| Cell type     | Adenocarcinoma | 165 (72.1)        | 147 (75.0)           | 18 (54.5)  | 0.112 | |
|              | Squamous cell carcinoma | 45 (19.7)       | 34 (17.3)            | 11 (33.3)  | 0.040 | |
|              | NSCLC, others | 14 (6.1)               | 11 (5.6)             | 3 (9.1)    | 0.818 | |
|              | Small cell carcinoma | 5 (2.2)                      | 4 (2.0)              | 1 (3.0)    | 0.818 | |
| Smoking       | Smoker | 132 (57.6)            | 109 (55.6)           | 23 (69.7)  | 0.130 | |
|              | Never smoked | 97 (42.4)              | 87 (44.4)            | 10 (30.3)  | 0.466 | |
| T stage       | T1/2   | 110 (48.0)            | 93 (47.4)            | 17 (51.5)  | 0.665 | |
|              | T3/4   | 119 (52.0)            | 103 (52.6)           | 16 (48.5)  | 0.123 | |
| N stage       | N0/1   | 20 (8.7)              | 5 (2.61)             | 15 (45.5)  | <0.001 | |
|              | N2/3   | 209 (91.3)            | 191 (97.4)           | 18 (54.5)  | <0.001 | |
| M stage       | M0     | 60 (26.2)             | 35 (17.9)            | 25 (75.8)  | 0.002 | |
|              | M1a    | 52 (22.7)             | 50 (25.5)            | 2 (6.1)    | 0.198 | |
|              | M1b    | 117 (51.1)            | 111 (56.6)           | 6 (18.2)   | 0.198 | |

NSCLC = nonsmall cell lung cancer, SCN = supraclavicular lymph node, SD = standard deviation.

^ Mann–Whitney U test.

1 χ² test.

in the short-axis dimensions and investigated the CT and US imaging characteristics of SCNs (Figure 2). Final conclusions were reached by consensus.

The CT morphological characteristics included loss of the central fatty hilum, surrounding fat infiltration, conglomeration of >3 lymph nodes, and necrotic lower attenuation compared with the adjacent muscle of the target lymph node. The US morphological characteristics included loss of the central fatty hilum; absence of a central hiliar structure, which is a linear avascular hyperechoic area in the center of a lymph node, heterogeneous echo-texture which is when there are multiple small areas of varying echogenicity, lower echogenicity compared with the adjacent muscle, conglomeration of >3 lymph nodes (≥0.5 cm, respectively), eccentric cortical hyper-echo-hypoecho-texture which is when there are multiple small areas of varying echogenicity, lower echogenicity compared with the adjacent muscle, conglomeration of >3 lymph nodes (≥0.5 cm, respectively), eccentric cortical hypoechoic area in the center of a lymph node, and necrotic/cystic lower echogenic foci within the lymph node (Figure 3). These findings have been reported as imaging findings of a malignant lymph node.13–15

Formal radiologic reports before histologic diagnosis were reviewed.

FDG PET was performed using multislice PET/CT camera system, and for the calculation of SUV max, region of interest (ROI) of variable sizes was drawn on consecutive trans-axial slices around the SCN with increased 18F-FDG uptake. SUV max was calculated using the single maximum pixel count within the defined ROIs.

The US, CT findings, and the SUV max on FDG PET of the SCN were compared with those in the negative metastasis to find target SCNs for core biopsy. The receiver operating characteristic curve (ROC) was analyzed to evaluate the ideal cutoff value of the short-axis dimension of the SCN on CT and US, and the SUV max on FDG PET, to predict positive metastasis of the SCN. And the diagnostic performance of cutoff values in 3 modalities to predict positive metastasis of the SCN was evaluated.

Gene Analysis

EGFR mutation tests were performed by acquiring samples suitable for molecular analysis. We analyzed EGFR mutations for 151 feasible patients with adenocarcinoma in exons 18, 19, and 21 using the direct DNA sequencing method. Gene analysis was only performed once a patient regardless of sample source.

Biopsy-Related Complication

We evaluated the biopsy-related mortality and morbidity of the US-guided core biopsy of the SCN and the other biopsy methods including the percutaneous core needle and bronchoscopy-guided lung biopsy in the available patients regarding the primary cancer.

Statistical Analysis

To evaluate the consistency of the findings of the 2 raters, we calculated the interclass correlation coefficient (ICC). To explore the discriminating factors between the 2 groups, we used the χ² test, Fisher exact test, and Mann–Whitney U test in the univariate analysis. We applied the significant factors to the multivariable model in the univariate analysis. We selected the significant variables in the multivariable model using the backward elimination method. According to the univariable model, we calculated the area under the ROC to discriminate between the node-positive group and the node-negative group. The best cutoff was estimated which maximizes both sensitivity and specificity. All the data were analyzed using SPSS software (version 21.0; IBM SPSS, Armonk, NY).

RESULTS

Patient Characteristics

From January 2011 to December 2013, in the total 229 patients, the final diagnoses of primary cancer were...
adenocarcinoma ($n = 165$), squamous cell carcinoma ($n = 45$), other types of nonsmall cell lung cancer ($n = 14$), and small cell lung cancer ($n = 5$). There was no significant difference between the positive metastasis group and the negative metastasis group with respect to age, sex, cell type of the primary cancer, smoking history, and T stage. The positive metastasis group ($n = 196, 86\%$) was associated with higher N (N2/N3) and M stages ($P < 0.001$, respectively) (Table 1).

The true negative in the US-guided core biopsy was identified in 33 patients (86.8\%) among the 38 patients with initial negative US-guided core biopsy. Of the 229 specimens, 224 (97.8\%) were accurately diagnosed as either positive or negative for metastasis (true positive $= 191$, true negative $= 33$, false negative $= 5$, and false positive $= 0$). Overall, the sensitivity, specificity, and accuracy of US-guided core biopsy of SCN diagnosing metastasis were 97.5\%, 100.0\%, and 97.8\%, respectively. Five (2.6\%) false-negative patients had an increased size of SCN on follow-up CT, which suggested positive metastasis. Among them, metastasis was pathologically confirmed through a subsequent rebiopsy in 1 case.

**Imaging Analysis of the US, CT, and FDG PET**

The ICC regarding size was 0.811 to 0.880 on the US and CT (for the long-axis dimension on the CT, ICC $= 0.853$ [95\% confidence interval, CI: 0.809–0.887]; for the short-axis dimension on the CT, ICC $= 0.873$ [95\% CI: 0.835–0.902]; for the long-axis dimension on the US, ICC $= 0.811$ [95\% CI: 0.755–0.854]; and for the short-axis dimension on the US, ICC $= 0.880$ [95\% CI: 0.844–0.907]). The short-axis dimension of the SCNs measured on both CT and US was longer in the positive group.
than in the negative group (for all, \( P < 0.001 \)). The median SUV\(_{\text{max}}\) was higher in the positive metastasis group than the negative metastasis group (6.8 vs 2.8, \( P < 0.001 \)) (Table 2). As for the radiological findings, loss of the central fatty hilum of the SCN was the most sensitive radiological finding (71% on CT, 76% on US) (Table 3).

The cutoff value of the short-axis dimension of SCN on the CT was 0.85 cm (sensitivity, 72.3%; specificity, 80.6%; AUC, 0.808, 95% CI: 0.740–0.875), on the US 0.75 cm (sensitivity, 73.5%; specificity, 84.8%; AUC, 0.843, 95% CI: 0.788–0.897), and that of SUV\(_{\text{max}}\), 4.05 (sensitivity, 79.1%; specificity, 81.8%; AUC, 0.853, 95% CI: 0.780–0.925) (Figure 4).

The sensitivity and specificity of US according to the short-axis dimension of the SCNs were 98.5% and 9.7% in reference to 0.5 cm, and 65.6% and 90.3% in reference to 1.0 cm, respectively. The SUV\(_{\text{max}}\) of \( \geq 2.5 \) on FDG PET showed sensitivity of 92.9% and specificity of 42.4%. Those are the current reference standards in the routine practice of our

**FIGURE 2.** Measurement of the size of the SCN on the CT and the US. The short- and the long-axis dimensions (cm) are measured on (A) the CT and (B) the US in perpendicular angle. (C) Note the loss of normal central fatty hilum as suggestive of malignancy on US. (D) Images show US-guided core biopsy of the malignant-looking SCN after needle entry during procedure. (E) Photomicrograph of histologic specimen shows adenocarcinoma (hematoxylin and eosin stain, \( \times 40 \)). (F) The core biopsy specimen of SCN is positive for thyroid nuclear factor 1 of immunohistochemical stain. (G) And using SCN biopsy sample for DNA sequencing method, a deletion in exon 19 (del E746_A750, arrow) is detected. CT = computed tomography, SCN = supraclavicular lymph node, US = ultrasonography.
hospital. The combination of the cutoff value of 0.75 cm on US and the $SUV_{\text{max}}$ of 4.05 on the FDG PET was more reasonable (sensitivity, 90.8%; specificity, 69.7%) (see Supplementary Appendix 1, http://links.lww.com/MD/A341).

The diagnostic performance of the stepwise approach especially in the group with the high N and M stages is shown in Supplementary Appendix 2, http://links.lww.com/MD/A341, with Figure 5. We used the $SUV_{\text{max}}$ cutoff value of 2.5 (not 4.05), on the FDG PET, because in real clinical practice, raising the sensitivity could be more important so as not to miss the metastasis. The sensitivity of the cutoff value of 4.05 is only 79.1% compared with 92.9% in that of 2.5 on the FDG PET.

The SCN metastasis was not mentioned on the formal radiologic reports in 56 of 229 CT scans (24.5%) in whom it was identifiable on US and FDG PET, and among them, 35 of 56 patients (62.5%) were confirmed to have had SCN metastasis through additional US-guided core biopsy. The SCNs with metastasis were not measurable in 3 of initial 229 CT scans.

**Gene Analysis**

There was no significant difference in the EGFR mutation types of the SCN biopsy samples and the other samples (Table 4). The EGFR mutation in the SCN was analyzed only in 106 of 151 (70.2%) patients with adenocarcinoma. The EGFR mutations in the SCN biopsy sample were positive in 38 of the 106 patients (35.8%) with adenocarcinoma, and the EGFR mutations in the other samples (primary tumor, n = 29; metastasis, n = 16) were positive in 31.1%. The most frequent mutation in SCN biopsy sample was exon 19 in 38 patients (n = 27, 71%).

**TABLE 2.** Comparison of the Dimensions on CT and US and FDG PET Findings Between the Positive Metastasis and the Negative Metastasis in the Supraclavicular Lymph Node

| SCN Biopsy, Median (IQR)† | Total | Positive Metastasis (n = 196, %) | Negative Metastasis (n = 33, %) | P       |
|---------------------------|-------|---------------------------------|-------------------------------|---------|
| CT-short-axis dimension²  | 1.0 (0.8, 1.4) | 1.0 (0.8, 1.5) | 0.7 (0.6, 0.8) | <0.001¹ |
| US-short-axis dimension    | 0.9 (0.7, 1.2) | 1.0 (0.7, 1.3) | 0.6 (0.5, 0.7) | <0.001¹ |
| FDG PET ($SUV_{\text{max}}$) | 6.1 (3.6, 8.9) | 6.8 (4.3, 9.8) | 2.8 (1.7, 3.5) | <0.001¹ |

CT = computed tomography, FDG PET = 18F-fluorodeoxyglucose positron emission tomography, SCN = supraclavicular lymph node, US = ultrasonography.

² Short-axis dimension.

¹ Interquartile range (lower first quartile: 25%, upper third quartile: 75%).

† Mann–Whitney $U$ test.

‡ For CT dimension, the total numbers differ due to nonmeasurable cases on the CT (positive metastasis, n = 195; negative metastasis, n = 31).

§ Maximal standardized uptake value.
TABLE 3. Comparison of the Morphologies of the Positive Metastasis and the Negative Metastasis in the Supraclavicular Lymph Node on US and CT

| CT Findings of the SCN | Positive Metastasis (Sensitivity, %) | Negative Metastasis (Specificity, %) |
|------------------------|-------------------------------------|-------------------------------------|
| Loss of the central fatty hilum | 139 (71.3) | 10 (67.7) |
| Conglomeration (≥3 lymph nodes) | 115 (59.0) | 4 (87.1) |
| Fat infiltration | 40 (20.5) | 0 (100.0) |
| Necrotic lower attenuation | 28 (14.4) | 1 (96.8) |
| Enhancement | 17 (8.9) | 1 (96.6) |

| US Findings of the SCN | Positive Metastasis (Sensitivity, %) | Negative Metastasis (Specificity, %) |
|------------------------|-------------------------------------|-------------------------------------|
| Loss of the central fatty hilum | 148 (75.5) | 8 (75.8) |
| Heterogeneous echotexture | 129 (65.8) | 11 (66.7) |
| Lower echogenicity | 127 (64.8) | 16 (51.5) |
| Conglomeration (≥3 lymph nodes) | 101 (51.5) | 3 (90.9) |
| Eccentric cortical hypertrophy | 96 (49.0) | 5 (84.8) |
| Necrotic/cystic lower echoic foci | 10 (5.3) | 1 (97.0) |

CT = computed tomography, SCN = supraclavicular lymph node, US = ultrasonography.

For CT findings, the total numbers differ due to nonmeasurable cases on the CT (positive metastasis, n = 195; negative metastasis, n = 31).

Compared with the adjacent muscle.

Biopsy-Related Complications

None of the patients had a complication after their US-guided core biopsy of the SCN. Of the 147 patients who underwent other biopsy procedures for intrathoracic lung cancer, including percutaneous core needle biopsy (n = 44) and bronchoscopy-guided biopsy (n = 34), 3 patients developed pneumothorax and 10 patients experienced bleeding after their percutaneous core needle biopsy, and 6 patients had tumoral bleeding and hemoptysis after their bronchoscopy-guided biopsy. The rate of mild to moderate complications after the biopsy of the main tumor was 12.9% (19/147). There was no life-threatening major complication.

DISCUSSION

Our findings suggested that US-guided core biopsy for SCN is a safe and reliable method of tissue confirmation showing high diagnostic accuracy (97.8%). According to our study, with the combination of the cutoff values of 0.85 cm short-axis dimension on CT, clinically used SUVmax 2.5 on the FDG PET, and 0.75 cm short-axis dimension on US, the specificity for SCN metastasis was reasonably high in any clinical stage and up to 100% in the M1 or N2/3. And also to appropriately select the target lymph node, morphologic characteristics of SCN metastasis including loss of the central fatty hilum, surrounding fat infiltration, conglomeration, and lower attenuation than muscle of the lymph node were not only subjective but also very important to reach high diagnostic accuracy.

Currently, chest radiologists have no reference standards to determine criteria for staging of lung cancer by size of SCN metastasis seen on initial CT. Some radiologists have reported possible metastasis in the SCN for a short-axis dimension of ≥0.5 cm, and others have reported possible metastasis only when the short-axis dimension is ≥1.0 cm. However, the cutoff values of 1.0 or 0.5 cm seem inappropriate because we found these values are either too specific or too sensitive. The cutoff values of a short-axis dimension of 0.85 cm on CT and 0.75 cm on US suggested herein can be used for greater diagnostic accuracy to predict SCN metastasis. The cutoff value of SUVmax on FDG PET is 4.05 in the present study. However, a SUVmax ≥ 2.5 on FDG PET has been widely used to increase the sensitivity. Metastasis in the SCN can be predicted in US-guided core biopsy by a larger size, a higher SUVmax, and an N2/3 or M1 stage of the underlying lung cancer.

The SCN metastasis was not mentioned in about one-quarter of our formal CT reports (56 patients, 24.5%), and among these, SCNs in 3 patients are barely measurable on CT in our study. The SCN metastases were variously too small to measure (<0.5 cm), or could not be measured because of a partial volume effect or volume averaging, or beam-hardening artifacts from bone or intravascular contrast material in the brachiocephalic veins.

Even though US-guided core biopsy gave a greater diagnostic yield, there were 5 false-negative patients (2.6%) in the present study. False-negative cases might be attributable to microscopic metastases or sampling error (only 1 to 2 cores) on initial US-guided SCN core biopsy rather than de novo metastasis within 6 months. Therefore, especially in N2/3 or M1 stage patients, a follow-up CT would be appropriate for an SCN with a negative biopsy result until no subsequent change is seen in at least the following 6 months. However, all N2/3 or M stage patients showed one or more malignant morphological characteristics of metastasis in the SCN on US. The morphological findings of SCN metastasis on images are subjective, but in our opinion, they are also important clues for the detection of metastases and should not be neglected.

Adopting a stepwise approach to evaluate SCN metastasis according to our routine clinical scenario, which starts with CT, FDG PET, and then US, especially in patients with an N2/3 or M1 stage, slightly decreases the sensitivity for detection because of the greater number of diagnostic methods, but dramatically increases the specificity by up to 100% through the combination of 3 methods. Our diagnostic approach to SCN metastasis is applicable to any clinical stage of lung cancer. Therefore, we recommend that in a diagnostic flowchart (Figure 5), US and US-guided core biopsy should be used routinely in the diagnostic work-up of patients suspected of...
having advanced lung cancer, and before any invasive procedure for primary cancer is undertaken.

Using tissue samples acquired from SCN core biopsies found a positive rate of EGFR mutation (35.8%) consistent with the results of previous studies that used other sampling methods for advanced adenocarcinoma. EGFR mutations are detected in approximately 40% of Korean nonsmall cell lung cancer patients with adenocarcinoma histology, and sample sources of primary and metastatic sites are equally suitable. Faster analysis confirming mutations in SCN samples could facilitate more timely targeted therapy of patients, especially those with symptomatic advanced lung cancers or initially palliative settings. Patients who have symptomatic chronic obstructive pulmonary disease with severe bulla or emphysema, who have usual interstitial pneumonia with little functional reservoir, or who have de novo lung cancer after previous pulmonary resection are not rare in our routine practice. An invasive method for pathological confirmation of intrathoracic cancer simultaneously burdens the patients, clinicians, and interventional radiologists.

We found no procedure-related morbidity in the present study of patients who underwent US-guided core biopsy, compared with those who underwent a more invasive biopsy procedure for primary cancers of the thorax (12.9%). There were no complications including catastrophic bleeding or vessel injuries in the present study after real-time US-guided core needle biopsy of the SCN. Nevertheless, a well-trained and experienced radiologist should perform the procedure carefully.

The present study has several limitations. First, it was a retrospective study performed at a single tertiary referral center. This may bias sampling of patients who consecutively underwent US-guided core biopsy for supraclavicular lymphadenopathy. The study does not evaluate the overall group of lung cancer patients with true- or false-positive CT scans for metastatic disease in the SCN. Second, we had a relatively small number of patients in the group with negative SCN metastasis for comparison, and further prospective study is needed to compare these patients with those having metastases. Third, there could have been errors in short-axis measurements, especially for metastases <0.5 cm. In the supravacular area, beam-hardening artifacts made it difficult to evaluate pathological lymph nodes by CT. The size of the lymph node could be overestimated by CT if there were conglomerations of lymph nodes or because of the partial volume effect and volume averaging. We measured long and short dimension at the accessible plane in each modality. The transverse scan of the body plane by US does not correspond precisely with the axial and coronal CT scan. However, concordance of the short- and long-axis dimensions as measured by CT and US by 2 chest radiologists in this study was excellent. Thin-section reconstruction and multiplanar reformatting of CT may improve the diagnostic value of lymph node measurements in future studies. Fourth, we could not compare the EGFR mutation status of the cancer in the primary site with that found in the SCN for confirmation of metastasis. Because of the clinical setting and cost, simultaneous confirmation of EGFR mutation in the apparent SCN metastasis and the primary cancer was not feasible in any patient. The concordance of EGFR mutations in primary tumor and metastases is 94%, and that for mutation ratios is 84%. Different types of mutations, such as those in exons 19 and 21, were also identified with high concordance, suggesting that the type of mutation did not affect the detection rates. In previous study, EGFR mutation status of metastatic lymph node is a predictive marker for the response to EGFR-tyrosine kinase inhibitor therapy; so even though we did

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FIGURE 4. The area under the receiver operating characteristic curve for distinguishing the patients with SCN metastasis using (A) CT, (B) FDG PET, and (C) US. CT = computed tomography, FDG PET = 18F-fluorodeoxyglucose positron emission tomography, SCN = supraclavicular lymph node, US = ultrasonography.
not confirm the EGFR mutation status of the primary cancer, the importance of EGFR mutation analysis for the SCN sample is not diminished.21 Finally, some discrepancy in determining the target lymph node occurred between the radiologist and the interventionist performing the US-guided biopsy when there were several enlarged lymph nodes in a single nodal station. Despite their limitations, SCN core biopsies by radiologists may be an important contribution to lung cancer staging, and mutation studies which have become routine for all lung adenocarcinoma patients. Because there has been limited information in this area, our study provides evidence-based rationale for US-guided SCN core biopsy in lung cancer patients.

**FIGURE 5.** Flow diagram demonstrating the patient’s diagnostic process for histologic subtyping and EGFR analysis regarding adenocarcinoma. Patient who has an enlarged SCN visible on initial chest CT >0.85 cm in the short-axis dimension undergo US-guided core biopsy of SCN. Patient undergoes FDG PET for the further analysis and if patient has a hypermetabolic SCN (SUV\text{max} ≥ 2.5), the US-guided core biopsy of SCN is firstly recommended. If there are no enlarged SCNs on the chest CT or no hypermetabolic SCNs, the next step depends on whether the stage is resectable or unresectable. In the former cases, tissue confirmation for primary tumor is necessary. Meanwhile, in the latter cases, US evaluation of the supravaculicular area is recommended. CT = computed tomography, EGFR = epidermal growth factor receptor mutation, FDG PET = 18F-fluorodeoxyglucose positron emission tomography, SCN = supravaculicular lymph node, SUV\text{max} = maximum standardized uptake value, US = ultrasonography.

**TABLE 4.** EGFR Mutation Analysis in the 151 Among 165 Adenocarcinomas

| EGFR Mutation | Total N = 151 (%) | SCN Biopsy N = 106 (%) | Other Samples\(^1\), N = 45, Primary Lung Biopsy, Metastasis Biopsy | \( P \) |
|---------------|-------------------|-----------------------|---------------------------------------------------------------|---|
| Exon 18       |                   |                       |                                                               |   |
| Positive      | 1 (0.7)           | 1 (0.9)               | 0 (0.0)                                                       | 0 (0.0) | 0 (0.0) | 0.871\(^2\) |
| Negative      | 150 (99.3)        | 105 (99.1)            | 22 (100)                                                      | 7 (100) | 16 (100) |             |
| Exon 19       |                   |                       |                                                               |   |
| Positive      | 34 (22.5)         | 27 (25.5)             | 4 (18.2)                                                      | 2 (28.6) | 1 (6.3) | 0.744\(^2\) |
| Negative      | 117 (77.2)        | 79 (74.5)             | 18 (81.8)                                                     | 5 (71.4) | 15 (93.8) |             |
| Exon21        |                   |                       |                                                               |   |
| Positive      | 17 (11.4)         | 10 (9.4)              | 5 (22.7)                                                      | 0 (0.0) | 2 (12.5) | 0.124\(^2\) |
| Negative      | 134 (88.7)        | 96 (90.6)             | 17 (77.3)                                                     | 7 (100) | 14 (87.5) |             |
| Total positive| 52 (34.4)         | 38 (35.8)             | 14 (31.1)                                                     |   |

EGFR = epidermal growth factor receptor, PCN = percutaneous core needle lung biopsy, SCN = supravacular lymph node, TBLB = transbronchial lung biopsy.

\(^a\) In 14 of 165 adenocarcinoma patients, EGFR studies were not performed due to various causes including economic status of patients or clinical decision.

\(^b\) Other sample = from a primary lung tumor or metastasis in other organs (percutaneous core needle lung biopsy, n = 22; transbronchial lung biopsy, n = 7; transbronchial lymph node biopsy, n = 13; distant metastasis biopsy including adrenal gland, liver and diaphragm, n = 3).

\(^c\) Fisher exact test.
findings are immediately applicable in routine radiological and clinical practice using a multidisciplinary approach.

CONCLUSIONS

US-guided SCN core biopsy is a reliable and safe method of evaluating the presence of metastasis, for histologic subtyping, and for investigation of mutations in advanced lung cancer. It may be a substitute for more invasive lung biopsy as an initial tissue confirmation in the advanced disease.

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