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The Macroscopic Appearance of Computed Tomography-guided Needle Biopsy Specimens Correlates with Tumor Metastasis in Non-small Cell Lung Cancer

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Abstract

Background

Computed tomography (CT)-guided needle biopsy is a well-established and dependable procedure for the diagnosis of pulmonary lesions. Some tissue biopsy samples have loose cohesion and disintegrate into tiny pieces before formalin fixation. The purpose of this study was to assess the association between the fresh macroscopic appearance of samples obtained using CT-guided needle biopsy and the clinicopathological features of non-small cell lung cancer (NSCLC).

Methods

A total of 111 patients who underwent CT-guided lung needle biopsy at Osaka City University Hospital between May 2009 and May 2013 were enrolled. Macroscopic appearance was categorized as either loose or tight cohesion. Samples were evaluated using Azan staining to detect collagen fibers. The staining intensity was multiplied by the percentage of positive cells, and the specimen was categorized as having either low (<100) or high expression ($\geqq 100$). Univariate and multivariate logistic regression models were used to evaluate significant covariates for tumor metastasis.

Results

In the cohort of 111 patients, the diagnostic rates in loose and tight cohesions were 82.6% and 87.5%, respectively ($p=0.509$). In 60 patients diagnosed with NSCLC, Azan staining of collagen fibers was positive in 93.5% of the samples with tight cohesion and 28.6% of the samples with loose cohesion ($p<0.001$). In the multivariate logistic regression models, distant metastasis was significantly associated with loose cohesion ($p=0.026$).

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Conclusions

These results suggest that the macroscopic appearance of CT-guided biopsy samples correlates with tumor metastasis in NSCLC.

Key Words: Non-small cell lung cancer; Computed tomography (CT)-guided needle biopsy; Tumor metastasis

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. According to the World Health Organization (WHO) classification, non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases[1]. Difficulty in early detection by chest radiography, highly invasive properties, and frequent distant metastasis contribute to the poor prognosis of NSCLC. To improve prognosis, lung cancer screening programs for early detection using low-dose computed tomography (LDCT) are now widely used. However, tumors detected by LDCT are too small to sample using a bronchoscopic biopsy. CT-guided needle biopsy is a well-established and reliable procedure for diagnosis of pulmonary lesions owing to its high accuracy (95%), sensitivity (93%), and specificity (100%)[2], and is a useful tool in the diagnosis of lung nodules measuring 1.0 cm or smaller[3]. Previously, we reported an oblique approach for difficult cases[4]. This method is useful for small targets, as well as those located under a rib. Our method allows a trajectory to be planned that avoids bones, bullae, fissures, and vessels. CT-guided needle biopsy is now an essential tool in characterizing lung abnormalities.

CT-guided needle biopsy samples obtained from small lesions may exhibit poor cohesiveness and disintegrate into tiny pieces before formalin fixation. It is important to establish whether pathologists can make a histological diagnosis using the loose cohesion sample. In addition, the macroscopic appearance of fresh specimens with respect to loose or tight cohesion may correlate with pathological fibrotic change, thereby predicting clinical stages in NSCLC patients. We conducted a study to examine the association of macroscopic appearance with clinical stage, pathological differentiation, and state of fibrotic change.

Materials and methods

Patients

Patients who underwent CT-guided lung needle biopsy at Osaka City University Hospital between May 2009 and May 2013 were enrolled in this study. All the participants provided written informed consent. Samples from patients diagnosed with NSCLC were evaluated using hematoxylin-eosin and Azan stainings. Clinical data was collected, including sex, age, comorbidities, histological type, degree of differentiation, and tumor size. Clinical TNM staging was evaluated by CT scans of the chest and abdomen, brain MRI, bone scintigraphy, to find distal metastasis and lymph node metastasis according to the seventh edition of the guidelines of the Union for International Cancer Control for TNM staging of lung cancer[5]. This study was registered in the University Hospital Medical Information Network Clinical Trials Registry (observational study ID: UMIN000013965).

Biopsy procedures

CT-guided core biopsies were performed by two or more pulmonologists and radiologists using a procedure similar to that previously reported[4]. Briefly, lesions were imaged on a CT scanner (CT-W2000AD, Hitachi, Tokyo, Japan) as 5-mm-thick contiguous axial tomographic sections, at 120 kVp and 100 mA, before CT-guided lung needle biopsy. When a nodule was detected, preliminary helical

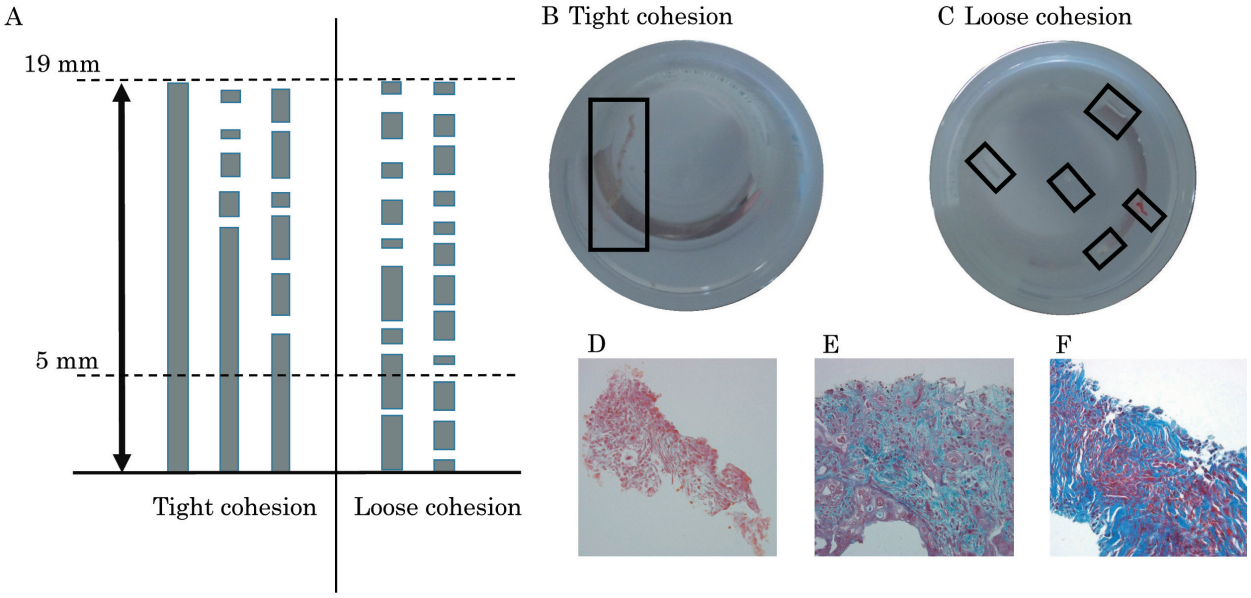

Figure 1. Categorization of cohesion. The left cartoon shows the definition of two groups (A). The right upper photos show an example of cohesion (B, tight cohesion; C, loose cohesion). The obtained samples were framed with a slit box (B) or small boxes (C). The right lower photos show examples of intensity of Azan staining (D, 1+; E, 2+; F, 3+), which magnification was ×200.

CT images were obtained in 3-mm-thick sections through the lesion. All the biopsies were performed twice shots with an 18-gauge introducer needle (needle length, 100 mm; Hakko, Tokyo, Japan) and a 20-gauge core tissue biopsy needle (needle length, 160 mm; Bard, Covington, LA, USA) using a Bard Magnum biopsy instrument (Bard). Before formalin fixation, two investigators scored the fresh macroscopic appearance of the tissue specimen (19 mm×0.6 mm×0.1 mm) as loose or tight cohesion. Tight cohesion was defined when the maximum tissue section was longer than 5.0 mm macroscopically. Loose cohesion was defined when the maximum tissue section was shorter than 5.0 mm macroscopically (Fig. 1).

Histology studies

The tumor specimens were fixed in 10% neutral formalin and embedded in paraffin, and 4 μm-thick serial sections were prepared. After dewaxing in xylene and rehydrating stepwise in ethanol, hematoxylin-eosin staining was performed. In order to detect collagen fibers, Azan staining was performed using a previously described method[6], and evaluated independently by two investigators. The scoring index (0-300) was obtained as the product of the percentage and intensity of the immunostained sections, according to the method of Miao et al, with slight modifications[7]. In brief, the tissue fibrotic change was evaluated as the percentage of the area that showed positive staining (0%-100%), and the modal intensity of the positively stained cells was determined on a scale of 0 to 3+ (0, complete absence of staining; 1+, staining weaker than that of the normal bronchial epithelium; 2+, staining similar to that of the normal bronchial epithelium; and 3+, staining clearly more intense than that of the normal bronchial epithelium). Specimens were classified as either low (<100) or high expression (≧100) for each target.

Statistical analysis

Correlations between clinicopathological factors were evaluated using the χ^2 test. Relationships between dichotomous variables were evaluated using the Fisher exact test. Unconditional logistic

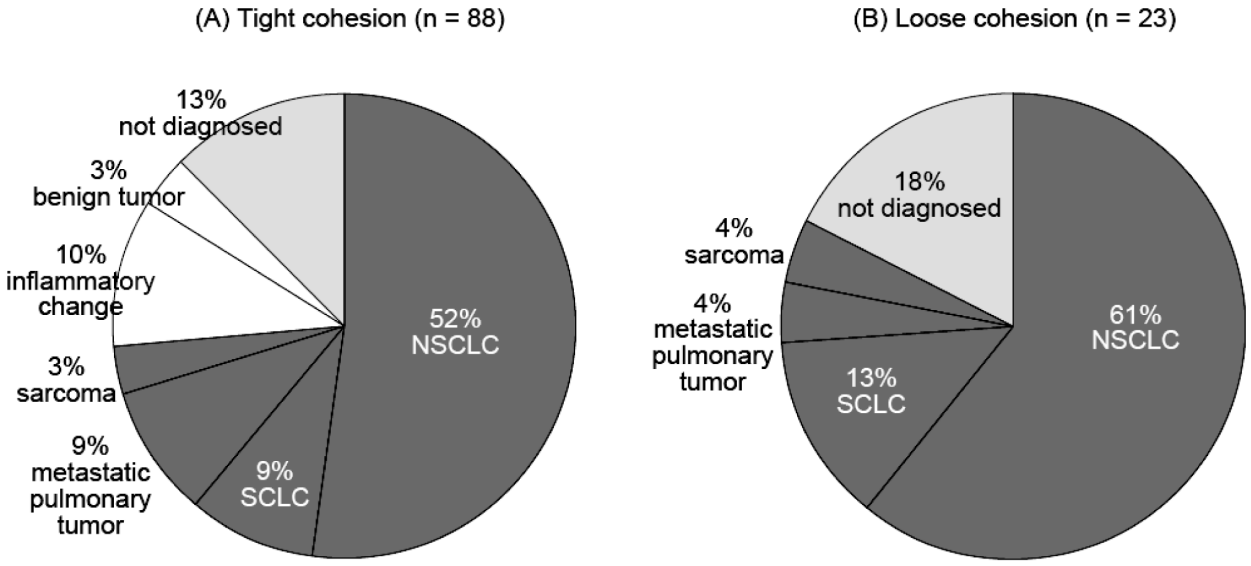

Figure 2. Diagnostic rates in tissues with tight loose cohesion. Malignant disease (dark grey area), benign disease (white area), and not diagnosed (light grey area).

regression was used to compute the odds ratios (ORs) and their 95% confidence intervals (CIs) for distant or lymph node metastasis based on sex, age, histological pattern, degree of differentiation, tumor size, Azan staining, and macroscopic appearance. Multivariate logistic regression models were used to investigate significant covariates for distant or lymph node metastasis based on sex, age, histological pattern, degree of differentiation, tumor size, and macroscopic appearance. All the statistical tests were two-sided. A $p<0.05$ was considered significant. All the statistical analyses were performed using JMP 9 software (SAS Institute, Inc., Cary, NC, USA).

Results

Patients characteristics

We recruited 111 patients aged between 39 and 87 years (median age, 71.0 years). On macroscopic examination, 20.7% specimens (n=23) had loose cohesion and 79.3% (n=88) had tight cohesion (Fig. 2). Ninety-six (86.5%) of the 111 biopsy samples had adequate material for diagnosis. The diagnostic rates in tight and loose cohesions were not significantly different ($p=0.509$) at 87.5% (77/88) and 82.6% (19/23), respectively. Of the 77 samples with tight cohesion, 46 patients were diagnosed with NSCLC; 8, with small cell carcinoma of the lung; 8, with metastatic tumors; 9, with inflammatory change; 3, with benign tumors; and 3, with sarcomas. For the 19 samples with loose cohesion, the following diagnoses were made: NSCLC in 14 patients, small cell carcinoma of the lung in 3 patients, metastatic tumor in 1 patient, and sarcomas in 1 patient.

Association between macroscopic appearance and clinicopathological markers in NSCLC

The NSCLC patients' characteristics are shown in Table 1. Sixty patients diagnosed with NSCLC were evaluated for fibrosis using Azan staining. No significant association was found between the macroscopic appearance of the tissue specimen and the clinicopathological features, including sex, age, histological type, degree of differentiation, and tumor size ($p=0.483$, $p=1.0$, $p=0.710$, $p=0.356$, and $p=0.140$, respectively). Azan staining of collagen fibers was positive in 93.5% of the samples with tight cohesion and 28.6% of the samples with loose cohesion ($p<0.001$).

Table 1. The characteristics of the patients with NSCLC

Clinicopathological feature	Tight cohesion (n=46)	Loose cohesion (n=14)	p value
Sex			
Male/Female	33/13	12/2	0.483
Age, years			
≧70/<70	30/16	9/5	1.000
Histological type			
Sq/Ad/other	11/28/7	2/8/4	0.710
Differentiation			
Well/Moderate/Poor	18/12/16	4/3/7	0.586
Tumor size			
Large (≧20 mm)/Small (<19 mm)	22/24	10/4	0.140
CT imagings			
Solitary nodules/Ground glass nodule	44/2	13/1	0.140
Azan staining			
≧100/<100	43/3	4/10	<0.001
Lymph node metastasis			
Positive/Negative	20/26	10/4	0.125
Distal metastasis			
Positive/Negative	10/36	8/6	0.019

NSCLC, non-small cell lung cancer; sq, squamous cell carcinoma; and ad, adenocarcinoma.

Prognostic factors

Forest plots of the odds ratio for distant and lymph node metastases are shown in Figure 3. In the univariate analysis, a macroscopic appearance of tight cohesion was significantly associated with distant metastasis (p=0.019; OR, 0.21; 95% CI, 0.06-0.73). Macroscopic appearance had no significant association with lymph node metastasis (p=0.125).

Multivariate logistic regression models were used to investigate significant covariates for distant and lymph node metastases according to sex, age, histological type, degree of differentiation, tumor size, and macroscopic appearance. Distant metastasis was significantly associated with macroscopic appearance (p=0.026). The association between lymph node metastasis and macroscopic appearance tended toward significance (p=0.054).

Discussion

Our study demonstrates an association between macroscopic appearance, and clinical stage, pathological differentiation, and fibrotic change status. Most of the specimens with loose cohesion (82.6%) provided adequate material for diagnosis. Of the specimens with tight cohesion, 12 (13.6%) were of benign disease, including 6 (6.8%) of old inflammatory nodules, 3 (3.4%) of interstitial pneumonitis, and 3 (3.4%) of granulomas. Benign nodules may have inflammatory cells, which may induce fibroblasts during the healing process[8]. Among the biopsy samples with loose cohesions, 14 (60.9%) were NSCLC and 5 (21.7%) were other types of malignant tumors, whereas 4 (17.4%) were not diagnosed. Therefore, samples with loose cohesion may have a high probability of indicating malignant disease.

In our study, tissue with loose cohesion was significantly associated with distant metastasis in the NSCLC patients (p=0.019). In the multivariate analysis, distant metastasis was significantly associated with macroscopic appearance. Moreover, low-level Azan staining of collagen fibers was

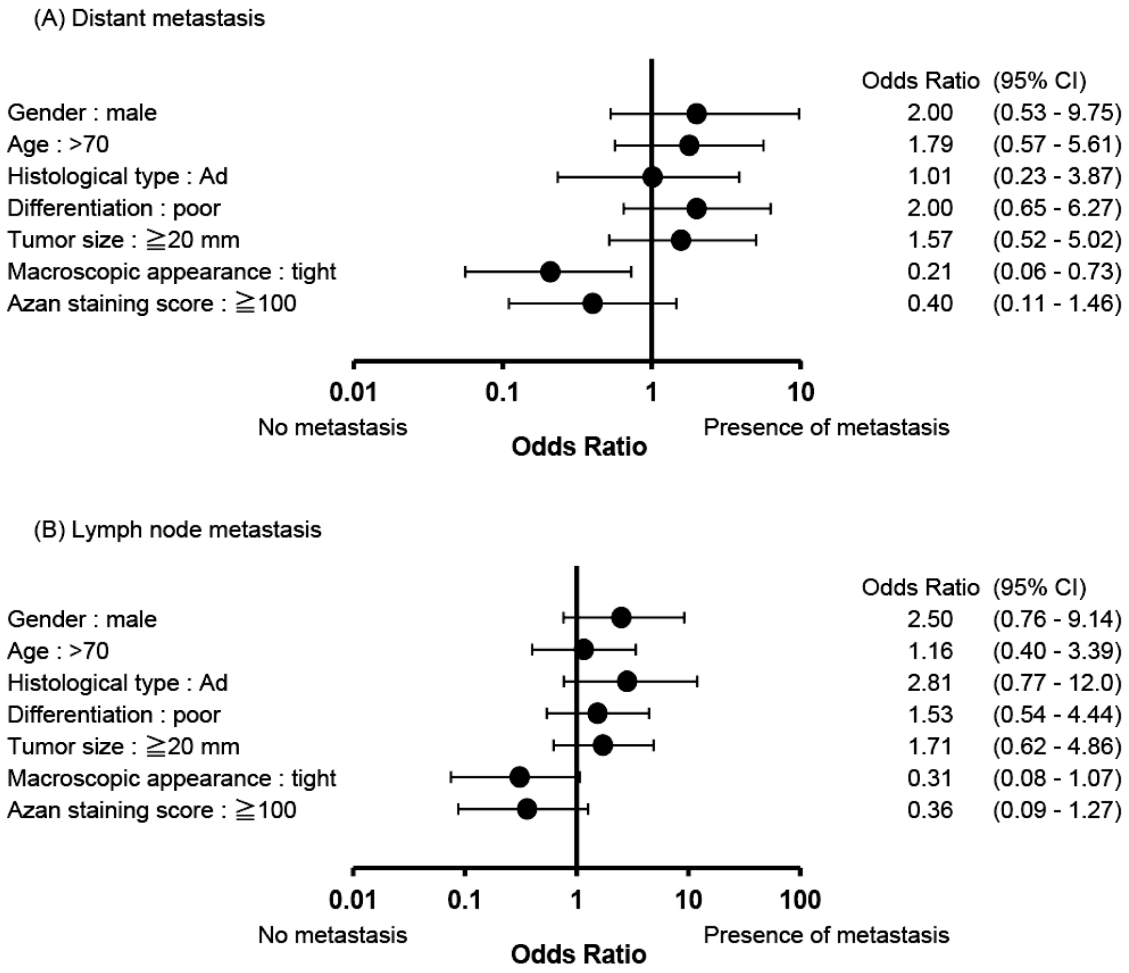

Figure 3. Forest plots of the odds ratio for distant and lymph node metastases. Multivariate logistic regression models were used to investigate significant covariates for distant (A) or lymph node (B) metastasis.

observed in 28.6% of the samples with loose cohesion. Our study indicates that tumors with loose cohesion may have relatively few fibers and loss of adhesion to adjacent cells, resulting in advanced clinical stages.

The International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS) /European Respiratory Society (ERS) proposed International Multidisciplinary Lung Adenocarcinoma Classification, using comprehensive histological subtyping with lepidic, acinar, papillary, solid, and micropapillary patterns.

The histological types were distributed into two prognostic groups according to grade as follows; intermediate-grade adenocarcinomas, corresponding to lepidic-predominant and acinar-predominant; and high-grade adenocarcinomas, corresponding to micropapillary-predominant, solid-predominant with mucin production and papillary-predominant adenocarcinoma[9,10]. In this classification, microadenocarcinoma grow with enlarged nuclear and interstitial infiltration, influencing disease prognosis[11].

In this classification, it is unclear the relationship between the cohensiveness conditions and the growth subtypes of adenocarcinoma. In our study, the loose cohesion specimen may be including micropapillary predominant pattern and papillary-prdominant adenocarcinomas proposed the new classification, and the tight cohesion may be including lepidic-predominant, acinar-predominant and

solid-predominant adenocarcinomas.

Because of small pieces, micropapillary predominant pattern have little collagen fibers, however, solid predominant pattern have rich collagen fibers in Azan staining, therefore our results are showen a new aspect of this new classification.

Although the whole tumor is obtained during surgery, its fresh macroscopic appearance cannot be easily evaluated because it is quickly preserved in formalin. In our study, macroscopic appearance was evaluated using a very thin stick column obtained using needle biopsy; this method has some merits. First, every stick specimen was the same size because an 18-gauge needle was inserted in all the target lesions. Second, the method is a convenient and quick diagnostic technique. In consequence, we could use macroscopic appearance as a predictive factor of distal metastasis before surgical staging.

This study had some limitations. The study population was a prospective cohort and was thus relatively small. We did not confirm the expression of EMT markers. Macroscopic appearance was subjectively evaluated by each investigator, which has the potential to introduce bias. We did not analyze small cell carcinoma of the lung. The question we have to ask here is whether over 5.0 mm lung alveoli in obtained biopsy sample will be classified as tight cohesion. When we shot normal lung areas accidentally, little samples were obtained. That is thought to be casually related to the lack of collagen fibers compared to tumor lesion. Therefore, in case of the target mixed with tumor and normal lung lesions, the judged samples obtained was composed of only tumor involvement. However, the macroscopic appearance of the tissue specimens provided information that predicted the clinical status of the patients with NSCLC. Conclusively, we found that specimens with loose cohesion could provide an adequate amount of samples for diagnosis. Macroscopic appearance before formalin fixation correlates with the amount of collagen fibers. Loosely cohesive specimens were correlated with tumor metastasis.

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