Ann Thorac Cardiovasc Surg 2015; 21: 102–108
Online June 3, 2014
doi: 10.5761/atcs.oa.14-00093

Original Article

Imaging Characteristics in *ALK* Fusion-Positive Lung Adenocarcinomas by Using HRCT

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Objectives: We aimed to identify high-resolution computed tomography (HRCT) features useful to distinguish the *anaplastic lymphoma kinase* gene *(ALK)* fusion-positive and negative lung adenocarcinomas.

Methods: We included 236 surgically resected adenocarcinoma lesions, which included 27 consecutive *ALK* fusion-positive (AP) lesions, 115 *epidermal growth factor receptor* mutation-positive lesions, and 94 double-negative lesions. HRCT parameters including size, air bronchograms, pleural indentation, spiculation, and tumor disappearance rate (TDR) were compared. In addition, prevalence of small lesions (≤20 mm) and solid lesions (TDR ≤20%) were compared.

Results: AP lesions were significantly smaller and had lower TDR (%) than *ALK* fusion-negative (AN) lesions (tumor diameter: 20.7 mm ± 14.1 mm vs. 27.4 mm ± 13.8 mm, respectively, $p < 0.01$; TDR: 22.8% ± 24.8% vs. 44.8% ± 33.2%, respectively, $p < 0.01$). All AP lesions >20 mm (n = 7, 25.9%) showed a solid pattern. Among all small lesions, AP lesions had lower TDR and more frequent spiculation than AN lesions ($p < 0.01$). Among solid lesions, AP lesions were smaller than AN lesions ($p = 0.01$).

Conclusion: AP lung lesions were significantly smaller and had a lower TDR than AN lesions. Spiculation was more frequent in small lesions. Non-solid >20 mm lesions may be *ALK* fusion-negative.

Keywords: *ALK* fusion-positive, lung cancer, adenocarcinoma, computed tomography, imaging

Introduction

Lung cancer is the most common cause of cancer deaths worldwide, with >1 million deaths occurring each year. Adenocarcinoma is the most common lung cancer type in both non-smokers and smokers. In recent years, a novel transforming fusion gene joining the *anaplastic lymphoma kinase* gene *(ALK)* has been detected in lung carcinomas.[1)] The *ALK* fusion-type tyrosine kinase is an oncoprotein found in 4%–5% of non-small cell lung cancers (NSCLC), and clinical trials of specific inhibitors of *ALK* for the treatment of such tumors are currently

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Received: March 23, 2014; Accepted: April 30, 2014
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underway.[2–5] Other investigations have compared the clinicopathological features of *ALK* fusion-positive and *epidermal growth factor receptor (EGFR)* mutation-positive lung carcinomas, indicating that non-exposure to cigarette smoke or light smoking exposure is a common clinical feature in both groups.

Recent advances in high-resolution computed tomography (HRCT) screening has improved the detection rates of small lung cancers, especially adenocarcinomas. In this study, we compared the CT appearance of adenocarcinoma lesions positive for *ALK* fusion-positive (AP) and negative (AN) lung adenocarcinomas.

Materials and Methods

Patients

This retrospective study was performed after approval from our institutional review board, which waived the need for obtaining consent. A total of 27 patients undergoing surgical resection of AP lesions were found on record from October 2004 to December 2010 at the Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, Japan. The pathological stage of each lesion was evaluated according to the current international TNM staging system and the World Health Organization classification. Smokers and non-smokers were categorized on the basis of the smoking history questionnaire. Smokers were further classified into three subgroups on the basis of the smoking index (SI), which was calculated as the product of the number of cigarettes smoked and inhaled years. Subgroups of patients included non-smokers (SI = 0), light smokers (SI <600), and heavy smokers (SI ≥600). We compared the surgically resected AN adenocarcinomas, which included 115 adenocarcinomas positive for *EGFR* mutations and 94 adenocarcinomas that were negative for both (wild-type; WT) from January 2009 to December 2010 in our hospital.

Detection of *ALK* and *EGFR* mutations

A highly-sensitive immunohistochemical method, the intercalated antibody-enhanced polymer (iAEP) method, has been developed that enables reliable immunohistochemistry-based detection of *ALK* fusion products. This technique can be applied to small biopsy samples, as those collected by endobronchial ultrasound-guided transbronchial needle aspiration.[6] We analyzed mutations in *EGFR* (exons 18–21) using the PCR-single strand conformational polymorphism technique.

Evaluation of tumor disappearance rate using multidetector CT

The CT images were retrieved on our Picture Archiving and Communication System (FUJIFILM Synapse 3.2.1 SR-356; FUJIFILM Corporation, Tokyo, Japan) and reviewed by members of our thoracic surgical oncology department in consensus. Chest images were acquired using multidetector CT with 5-mm slices. High-resolution images of targeted tumors were acquired using a 1.25-mm section thickness. Images on each sheet of film were photographed using mediastinal (level, 50 Hounsfield units [HU]; width, 400 HU) and lung (level, 600 HU; width, 1800 HU) window settings. Radiological parameters including size, air bronchograms, pleural indentation, spiculation, and tumor disappearance rate (TDR) on HRCT were analyzed for each patient. TDR ratios were defined as the ratio of the tumor area of the mediastinal window to that of the lung window on HRCT. TDR (%) was calculated as [1 – (maximum dimension of tumor in the mediastinal windows/ maximum dimension of tumor in the lung windows)] × 100.[7–10] We classified the pulmonary lesions into one of the three categories on the basis of TDR as follows: TDR ≥75%, least solid lesion; TDR ≤20%, solid lesion; and all others, partially solid lesions. Herein, we showed representative CT images of AP lesions in **Fig. 1**. We also examined small lesions (≤20 mm) and solid lesions (TDR ≤20%) with respect to radiological parameters including size, air bronchograms, pleural indentation, spiculation, and TDR. We further examined differences in imaging features by gene variations compared *anaplastic lymphoma kinase* with *EGFR tyrosine kinase*.

Statistical analysis

All data were analyzed using SPSS software (SPSS version 17.0J; SPSS Inc., Chicago, Illinois, USA). Comparisons between two groups were made using the Mann–Whitney U-test. Comparisons between ≥3 groups were made using the Kruskal–Wallis test. The Cox proportional hazards model was used for multivariate analyses. The effect of each variable in the model was expressed by the hazard ratio with a 95% confidence interval. p values <0.05 were considered significant.

Results

Patient characteristics

The mean ages for each group in years were as follows: AP, 57.7; EP, 70; WT, 64.3. The gender ratio (M:F)

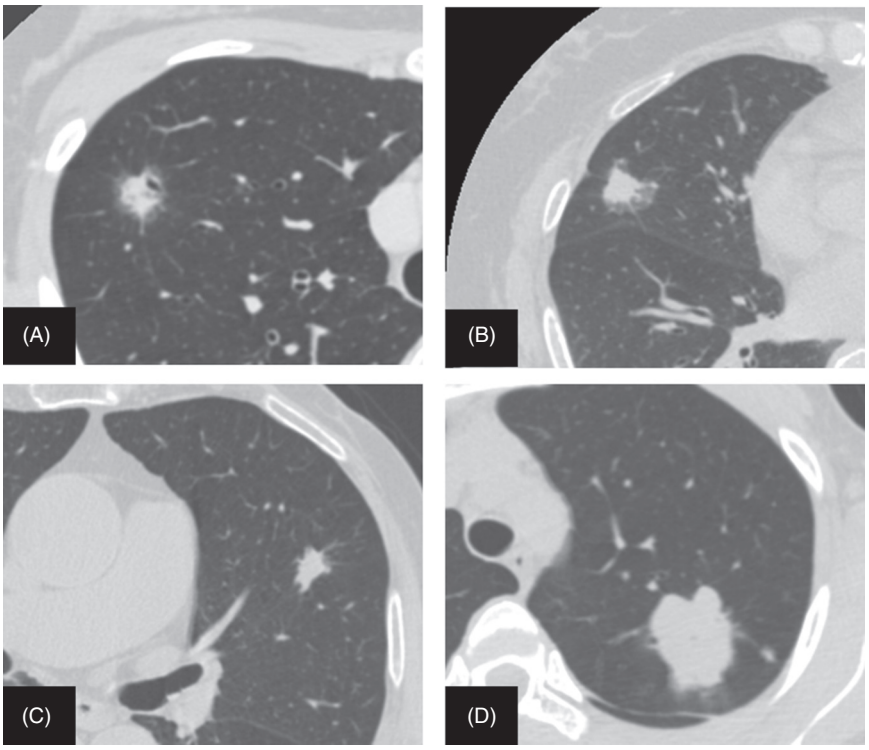

Fig. 1 High-resolution computed tomography images of *anaplastic lymphoma kinase (ALK)* fusion-positive lung cancer. (**A**) A 12-mm lesion with a tumor disappearance rate (TDR) of 25%, spiculation and air bronchogram. (**B**) A 17-mm lesion with a TDR of 35.3% and pleural indentation. (**C**) A 16-mm lesion with a TDR of 12.5% and spiculation. (**D**) A 42-mm lesion with a TDR of 4.8%.

in each group was as follows: AP, 8:19 (29.6% vs. 70.4%); EP, 28:87 (24.3% vs. 75.7%); WT, 55:39 females (58.5% vs. 41.5%). EP group mutations were in exon 18 (n = 3), exon 19 (n = 44), and exon 21 (n = 63). There were 5 patients with duplicative exons: 18/21 (n = 2), 19/20 (n = 1), 19/21 (n = 1), and 20/21 (n = 1). Of the 236 resected adenocarcinomas, 27 were AP lesions. The clinicopathological features of these lesions are summarized in **Table 1**. There were no *EGFR* mutation-positive AP lesions. AP lesions were more likely to be moderately/poorly differentiated and have positive lymph node metastasis than AN lesions (p <0.01; p = 0.01). AP lesions tended to occur in patients who were younger (p = 0.03), had lighter smoking exposure (p <0.01), and in women (p <0.01) compared with WT lesions. Age (p <0.01), smoking exposure (p <0.56), and gender (p <0.57) were the only variables that differed significantly between AP and EP lesions.

Radiological analysis of *ALK* fusion-positives

Table 2 shows the radiological features of pulmonary nodules from AP and AN lesions acquired using HRCT. The mean size was significantly lower in AP lesions (0.76-fold, 20.7 mm ± 14.1 mm) than in AN lesions (27.4 mm ± 13.8 mm) (p <0.01). AP lesion types comprised 15 (55.6%) solid, 10 (37.0%) partial solid, and five (7.4%) least solid lesions. AN lesion types comprised 64 (30.6%) solid, 93 (44.5%) partial solid, and 52 (24.9%) least solid lesions. All >20-mm AP nodules (n = 7, 25.9%) were of solid type. The mean TDR score was significantly higher (1.96-fold) in AN lesions (44.8 ± 33.2) than in AP lesions (22.8 ± 24.8) (p <0.01). The prevalence of air bronchograms, pleural indentation, and spiculation did not differ significantly between AN and AP lesions (p = 0.374, p = 0.487, p = 0.941, respectively).

Subtype categories (small size and solid-type pattern)

Small lung nodules (≤20 mm in the maximum dimension) comprised 74.1% (20/27) AP lesions and 36.3% (76/209) AN lesions; this difference was significant (p <0.01). No significant difference was observed in the mean lesion size (AP, 13.9 mm ± 3.8 mm; AN, 14.6 mm ± 3.5 mm; p = 0.47). The mean TDR was

Table 1 Clinicopathological characteristics of lung adenocarcinomas

	ALK	*EGFR*	WT
Number	27	115	94
Age (yr; mean ± SD)	57.7 ± 14.4	70.0 ± 10.1	64.3 ± 10.2
Sex			
Male	8 (29.6%)	28 (24.3%)	55 (58.5%)
Female	19 (70.4%)	87 (75.7%)	39 (41.5%)
Smoking index			
Never (SI = 0)	21 (77.8%)	85 (74.0%)	41 (44.6%)
Light (SI <600)	4 (14.8%)	17 (14.7%)	20 (21.3%)
Heavy (SI ≥600)	2 (7.4%)	13 (11.3%)	33 (35.1%)
Differentiation			
Well	5 (18.5%)	74 (64.3%)	51 (56.7%)
Moderate/Poor	22 (81.5%)	41 (35.7%)	39 (43.3%)
f-stage			
IA	14 (51.9%)	69 (60.0%)	53 (56.4%)
IB	1 (3.7%)	19 (16.5%)	23 (24.5%)
II–IV	12 (44.4%)	27 (23.5%)	18 (18.9%)

Smoking index = (number of cigarettes smoked per day) × (number of years smoked). *ALK*: *anaplastic lymphoma kinase*; *EGFR*: *epidermal growth factor receptor*; WT: wild-type; yr: year old; SD: standard deviation; SI: smoking index; f-stage: final stage

Table 2 Imaging characteristics of *ALK* fusion-positive and negative nodules on HRCT

	ALK fusion-positive	*ALK* fusion-negative	*p*-value
Total number	27	209	
Size (mm), (range)	20.7 ± 14.1 (6–71)	27.4 ± 13.8 (6–100)	p <0.01
≤20	20 (74.1%)	76 (36.4%)	p <0.01
>20	7 (25.9%)	133 (63.6%)	p <0.01
Air bronchogram	19 (70.4%)	156 (74.6%)	p = 0.374
Pleural indentation	19 (70.4%)	141 (67.5%)	p = 0.487
Spiculation	22 (81.5%)	156 (74.6%)	p = 0.941
TDR (%)	22.8 ± 24.8	44.8 ± 33.2	p <0.01
Solid (TDR ≤20%)	15 (55.6%)	64 (30.6%)	p = 0.01
Partial solid (20< TDR <75%)	10 (37.0%)	93 (44.5%)	p = 0.46
Least solid (TDR ≥75%)	2 (7.4%)	52 (24.9%)	p = 0.04

TDR (%) = [1 – (maximum dimension of tumor in the mediastinal windows/ maximum dimension of tumor in the lung windows)] × 100. *ALK*: *anaplastic lymphoma kinase*; HRCT: high-resolution computed tomography; TDR: tumor disappearance rate

significantly lower in AP lesions than in AN lesions (AP, 29.4% ± 25.6%; AN, 58.7% ± 33.8%; p <0.01). Spiculation was the only radiological feature that differed significantly between AP and AN lesions, occurring in 18 (90.0%) small AP nodules (p <0.01). Among sold type lesions, 15 were AP (55.6%) and 69 were AN (33%) lesions. AP lesions were significantly smaller than AN lesions (AP, 23.5 ± 11.4 mm; AN, 34.4 ± 12.8 mm; p = 0.01). The prevalence of air bronchograms (p = 0.32), pleural indentation (p = 0.84) and spiculation (p = 0.33) did not differ significantly between AP and AN lesions (**Table 3**).

ALK fusion-positive and *EGFR* mutation subtype

When the cutoff level of our graphic assay, which showed TDR on the y-axis and size on x-axis, was set as $y = -2x + 80$ according to the proportional formula, the proportion of AP lesions falling under this formula line was 88.9% (24/27) (**Fig. 2A**), whereas that for exon 19 *EGFR* mutation-positive lesions was 29.5% (13/44) (**Fig. 2B**) and for exon 21 *EGFR* mutation-positive lesions was 19.0% (12/63) (**Fig. 2C**); these differences were significant (exon 19 vs. AP, p <0.01; exon 21 vs. AP, p <0.01). The receiver operating characteristic curve was

Table 3 Imaging characteristics of *ALK* fusion-positive and negative nodules on HRCT (small size and solid-type pattern)

	ALK fusion-positive	*ALK* fusion-negative	*p*-value
Size ≤20 mm			
Total number	20 (74.1%)	76 (36.3%)	$p < 0.01$
Size (mm)	13.9 ± 3.8	14.6 ± 3.5	$p = 0.47$
Air bronchogram	13 (65.0%)	41 (53.9%)	$p = 0.38$
Pleural indentation	13 (65.0%)	37 (48.7%)	$p = 0.20$
Spiculation	18 (90.0%)	41 (53.9%)	$p < 0.01$
TDR (%)	29.4 ± 25.6	58.7 ± 33.8	$p < 0.01$
Solid (TDR ≤20%)	8 (40.0%)	12 (15.8%)	$p = 0.02$
Partial solid (20< TDR <75%)	10 (50.0%)	34 (44.7%)	$p = 0.68$
Least solid (TDR ≥75%)	2 (10.0%)	30 (39.5%)	$p = 0.01$
TDR ≤20%			
Total number	15 (55.6%)	66 (33%)	$p < 0.01$
Size (mm)	23.5 ± 11.4	34.4 ± 12.8	$p = 0.01$
Air bronchogram	10 (66.7%)	52 (78.9%)	$p = 0.32$
Pleural indentation	12 (80.0%)	58 (87.9%)	$p = 0.84$
Spiculation	12 (80.0%)	54 (81.2%)	$p = 0.33$

TDR (%) = [1 – (maximum dimension of tumor in the mediastinal windows/ maximum dimension of tumor in the lung windows)] × 100. *ALK*: *anaplastic lymphoma kinase*; HRCT: high-resolution computed tomography; TDR: tumor disappearance rate

shown in **Fig. 2D**. The area under the receiver operating characteristic curve for TDR in AP was 0.70.

Discussion

In an effort to develop new treatment strategies, several molecular-targeted drugs have recently been developed. Crizotinib was reported to cause tumor shrinkage or stabilization in about 90% patients positive for *ALK* fusion gene.[11] A phase 3 trial, PROFILE 1007, that compares crizotinib to standard second-line chemotherapy for *ALK*-positive NSCLC is presently underway. In addition, a phase 2 trial, PROFILE 1005, that studies patients meeting similar criteria who have received more than one line of previous chemotherapy is also in progress. To treat *ALK*-positive lung cancers, detection of this newly recognized fusion gene and its associated characteristics is important.

Several authors investigating *ALK* fusion-positive lung cancer reported the clinicopathological features such as lighter smoking exposure, younger age of onset, and no or rare mutations in *EGFR*, *KRAS*, and *TP53*. *EGFR* mutations have been reported more frequently in women, non-smokers, and Asian patients.[12–16] Common characteristics of our patients with *ALK* fusion-positive tumors included lighter smoking exposure, younger age, and female gender. Although light smoking exposure and female gender were common to both *ALK* fusion-positive and *EGFR* mutation-positive lesions in this study, AP lesions were associated with a lower patient age than EP lesions ($p < 0.01$). Pathologically, the presence of the solid signet-ring cell pattern or mucinous cribriform pattern can strongly predict *ALK* rearrangement status.[2–5] In this study, moderate to poorly differentiated adenocarcinoma was more strongly associated with *ALK* fusion-positive tumors than the others ($p < 0.01$).

To the best of our knowledge, this is the first report to study HRCT futures between the *ALK* fusion-positive and negative lung adenocarcinomas. We observed several significant differences between *ALK* fusion-positive tumors and the others. First, AP lesions were significantly smaller than AN lesions. Among AP tumors, 74.1% were ≤20 mm in size. Second, AP tumors showed a tendency toward the solid pattern because of their low TDRs. Radiological parameters including air bronchograms, pleural indentation, and spiculation did not differ significantly between tumor types. In this study, a 74-year-old female with a TDR of 100% and a 46-year-old male with a TDR of 88.9% were the cases with very high TDR, which was rare in the study population.

We categorized lesions by size (small size, ≤20 mm) and type (solid pattern, TDR ≤20%) on the basis of CT images. The majority of the tumors in the AP group were small. These small tumors had a solid pattern, which correlated with the fact that TDR was lowest in AP lesions. Spiculation was significantly more frequent in AP lesions

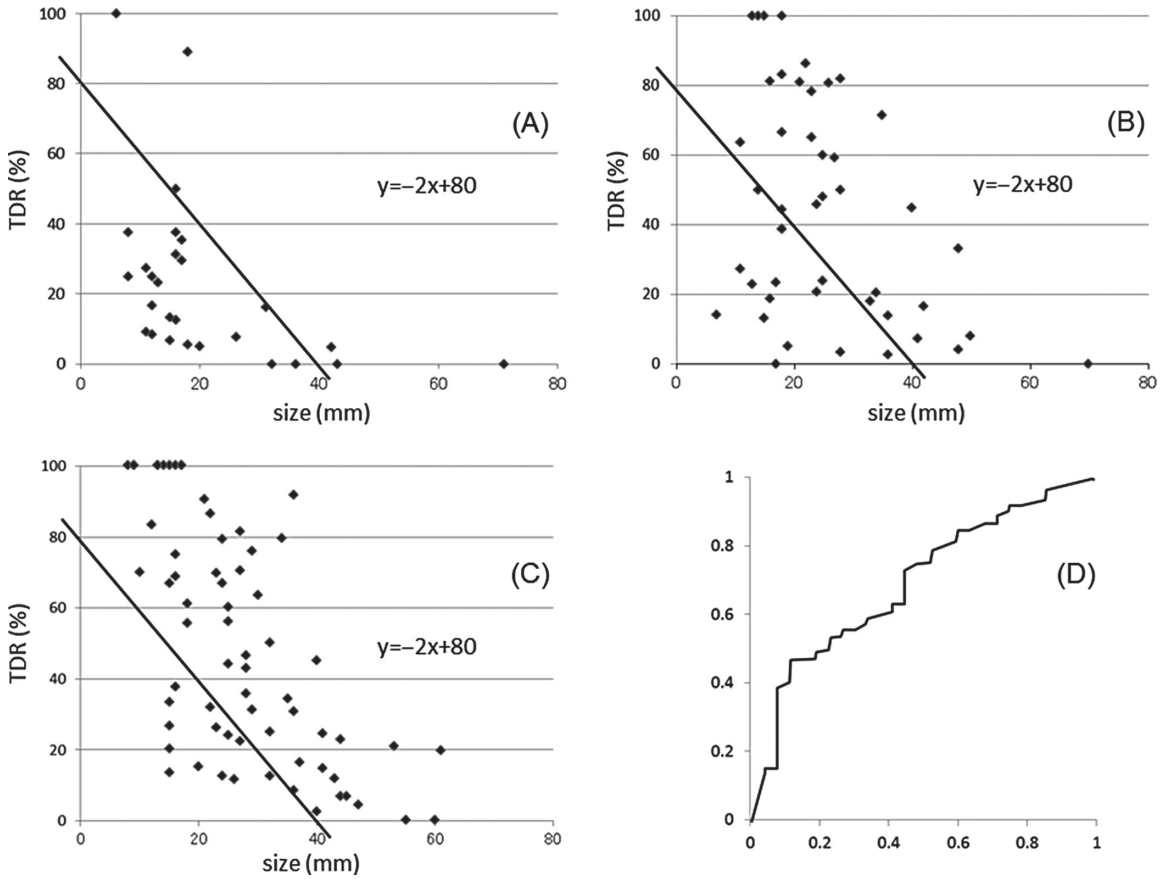

Fig. 2 When the cutoff level of our graphic assay was set as y = −2x + 80 according to the proportional formula, the number of anaplastic lymphoma kinase fusion-positive (AP) lesions falling under this formula line was 88.9% (24/27) (**A**), whereas that for exon 19 was 29.5% (13/44) (**B**) and for exon 21 was 19.0% (12/63) (**C**). Significant differences were found between exon 19 and AP lesions (p <0.01) and between exon 21 and AP lesions (p <0.01). The area under the receiver operating characteristic curve for tumor disappearance rate (TDR) in AP lesions was 0.70 (**D**).

than in AN lesions (p <0.01). Among solid-type tumors, AP lesions were significantly smaller than AN lesions (p = 0.01).

Overall, AP lesions were associated with a small tumor size and a solid tumor type with spiculation. In analysis comprising only solitary lesions, spiculation was not found to be significant predictive factor, and only small size remained a factor predictive of *ALK* fusion-positive lesions.

All seven AP lesions >20-mm diameter had a solid pattern. This subgroup had a significantly lower TDR than the group with smaller tumors (≤20 mm, p <0.01). Thus, our results suggest that lesions >20 mm diameter without a solid pattern may be negative for *ALK* rearrangement.

We further examined differences in imaging features by gene variations. In accordance with the fact that the *EGFR tyrosine kinase* and *anaplastic lymphoma kinase* have different characteristics, imaging of AP lesions was showed a limited distribution within the lung fields. In contrast, EP lesions showed extensively and diffuse distribution. So the limited part which its TDR were low and it was small width, was distributed over the relatively small zone.

If *ALK* mutation is predicted by CT findings before treatment, this may allow medical oncologists to consider the use of targeted therapies sooner in patients with advanced stage, tumor- related inadequate general condition or relapsed disease. In this study, we could detect several significant differences between tumors and the others by using HRCT to help suggest the *ALK* fusion-positive status. However, we could not found perfect CT characteristics. At this time we should investigate any pathological specimen according to the guideline to make a definite diagnosis.

Conclusion

ALK fusion-positive lung lesions were significantly smaller and had a lower TDR than *ALK* fusion-negative lesions. Spiculation was more likely to be present in small lesions. Lesions >20 mm without a solid pattern may be negative for *ALK* rearrangement.

Disclosure Statement

The authors declare that they have no conflict of interests.

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