verification cannot be done in clinical trials of Adj.C, as "surgery alone" can no longer be set as a reference due to ethical concerns.

Acknowledgments The statistical considerations were checked by Yasunori Satoh, Ph.D., Clinical Research Center, Chiba University Hospital.

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Original Research

LUNG CANCER

Appropriate Sublobar Resection Choice for Ground Glass Opacity-Dominant Clinical Stage IA Lung Adenocarcinoma

Wedge Resection or Segmentectomy

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Background: **The purpose of this multicenter study was to characterize ground glass opacity (GGO)-dominant clinical stage IA lung adenocarcinomas and evaluate prognosis of these tumors after sublobar resection, such as segmentectomy and wedge resection.**

Methods: **We evaluated 610 consecutive patients with clinical stage IA lung adenocarcinoma who underwent complete resection after preoperative high-resolution CT scanning and ^{18}F-fluorodeoxyglucose PET/CT scanning and revealed 239 (39.2%) that had a > 50% GGO component.**

Results: **GGO-dominant tumors rarely exhibited pathologic invasiveness, including lymphatic, vascular, or pleural invasion and lymph node metastasis. There was no significant difference in 3-year recurrence-free survival (RFS) among patients who underwent lobectomy (96.4%), segmentectomy (96.1%), and wedge resection (98.7%) of GGO-dominant tumors ($P = .44$). Furthermore, for GGO-dominant T1b tumors, 3-year RFS was similar in patients who underwent lobectomy (93.7%), segmentectomy (92.9%), and wedge resection (100%, $P = .66$). Two of 84 patients (2.4%) with GGO-dominant T1b tumors had lymph node metastasis. Multivariate Cox analysis showed that tumor size, maximum standardized uptake value on ^{18}F-fluorodeoxyglucose PET/CT scan, and surgical procedure did not affect RFS in GGO-dominant tumors.**

Conclusions: **GGO-dominant clinical stage IA lung adenocarcinomas are a uniform group of tumors that exhibit low-grade malignancy and have an extremely favorable prognosis. Patients with GGO-dominant clinical stage IA adenocarcinomas can be successfully treated with wedge resection of a T1a tumor and segmentectomy of a T1b tumor.** ***CHEST 2014; 145(1):66–71***

Abbreviations: FDG = ^{18}F-fluorodeoxyglucose; FOV = field of view; GGO = ground glass opacity; HRCT = high-resolution CT; HU = Hounsfield units; IRB = institutional review board; NSCLC = non-small cell lung cancer; OS = overall survival; RFS = recurrence-free survival; SUVmax = maximum standardized uptake value

Advances in radiologic techniques, such as high-resolution CT (HRCT) scanning and the widespread use of low-dose helical CT screening, have enabled frequent detection of early lung adenocarcinoma.[1-3] On HRCT scan, early lung adenocarcinoma often contains a nonsolid component, such as ground glass opacity (GGO), that is closely associated with a pathologic lepidic growth component.[4,5] Patients with GGO-dominant small lung adenocarcinoma are believed to have a good prognosis.[6] A recent study also demonstrated that patients with GGO-dominant clinical T1N0M0 lung adenocarcinoma (consolidation/tumor ratio ≤ 0.5 on thin-section CT scan) have an excellent prognosis after lobectomy.[7] Although patients with GGO-dominant tumors may be candidates for sublobar resection, there is no clear evidence to support this hypothesis.

For editorial comment see page 9

A prospective study that compared sublobar resection (wedge resection or segmentectomy) concomitant with lobectomy for clinical T1N0M0 non-small cell lung cancer (NSCLC) concluded that sublobar resection resulted in a high local recurrence and a low

survival rate.[8] However, sublobar resection for early lung cancer has been debated for a considerable amount of time. Several studies have demonstrated the usefulness of sublobar resection for peripheral small-sized NSCLC.[3,9-12] However, there currently is little evidence in patients who are optimal candidates for sublobar resection. Therefore, the present study aimed to characterize GGO-dominant clinical stage IA lung adenocarcinomas and to evaluate the prognosis of patients with these tumors after sublobar resection.

Materials and Methods

Patients

We evaluated the results of ^{18}F-fluorodeoxyglucose (FDG) PET/CT scans of 610 patients with clinical T1N0M0 stage IA lung adenocarcinoma from four institutions (Hiroshima University, Kanagawa Cancer Center, Cancer Institute Hospital, and Hyogo Cancer Center, Japan) between August 1, 2005, and June 30, 2010. Patients with incompletely resected tumors (R1 or R2) and those with multiple tumors or who had previously undergone lung surgeries were not included in our prospectively maintained database. Patient data obtained from this multicenter database were retrospectively analyzed for this study.

Patients underwent HRCT scanning and FDG-PET/CT scanning followed by curative R0 resection, and their tumors were staged according to the seventh edition of the *TNM Classification of Malignant Tumors*.[13] Mediastinoscopy or endobronchial ultrasonography was not routinely performed because all patients had undergone preoperative HRCT scanning and FDG-PET/CT scanning. HRCT scanning and FDG-PET/CT scanning revealed an absence of a >1 cm enlargement in mediastinal or hilar lymph nodes and an absence of >1.5 accumulation for the maximum standardized uptake value (SUVmax) in these lymph nodes, respectively. Sublobar resection was allowed in patients with complete disease removal as an optional procedure for a peripheral clinical T1N0M0 tumor that was intraoperatively assessed as N0 by frozen section evaluation of enlarged lymph nodes or by ensuring that there was no obvious enlargement of lymph nodes in the thoracic cavity. Systematic lymph node dissection, such as that of hilar and mediastinal nodes, was performed during segmentectomy but not during wedge resection. All patients showing pathologic lymph node metastasis received four cycles of platinum-based chemotherapy after surgery.

Manuscript received May 6, 2013; revision accepted July 12, 2013.
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Funding/Support: The authors have reported to *CHEST* that no funding was received for this study.
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DOI: 10.1378/chest.13-1094

The inclusion criteria were preoperative staging determined through HRCT scan and FDG-PET/CT scan, curative surgery without neoadjuvant chemotherapy or radiotherapy, and a definitive histopathologic diagnosis of lung adenocarcinoma. This study was approved by the institutional review boards (IRBs) of the participating institutions (Hiroshima University Hospital IRB, No. EKI-644; Kanagawa Cancer Center IRB, No. KEN-31; Cancer Institute Hospital IRB, No. 2008-1018; Hyogo Cancer Center IRB, No. H20-RK-15). The requirement of informed consent from individual patients was waived because this study was a retrospective review of a patient database.

HRCT Scanning

Sixteen-row multidetector CT scanning was used to independently acquire chest images of subsequent FDG-PET/CT image examinations. The following parameters were used to acquire high-resolution tumor images: 120 kVp, 200 mA, 1- to 2-mm section thickness, 512×512-pixel resolution, 0.5- to 1.0-s scanning time, high-spatial reconstruction algorithm with a 20-cm field of view (FOV), and mediastinal (level, 40 Hounsfield units [HU]; width, 400 HU) and lung (level, -600 HU; width, 1,600 HU) window settings. GGO was defined as a misty increase in lung attenuation without obscuring the underlying vascular markings. A GGO-dominant tumor was defined as having a $>50\%$ GGO component. We defined a solid tumor size as the maximum dimension of the solid component measured on lung window settings, excluding GGO.[14] CT scans were reviewed and tumor sizes determined by radiologists from each institution.

FDG-PET/CT Scanning

Patients were instructed to fast for ≥ 4 h before IV injection of 74 to 370 MBq FDG and were subsequently advised to rest for ≥ 1 h before FDG-PET/CT scanning. Blood glucose levels were determined before tracer injection to confirm a <150 mg/dL level. Patients with blood glucose levels of ≥ 150 mg/dL were excluded from imaging. For imaging, a Discovery ST (GE Healthcare), an Aquiduo (Toshiba Medical Systems Corporation), or a Biograph Sensation 16 (Siemens AG) integrated three-dimensional PET/CT scanner was used. Low-dose, nonenhanced CT images of 2- to 4-mm section thickness for attenuation correction and localization of lesions identified with PET scan were acquired from head to pelvic floor in each patient by standard protocol.

Immediately after CT imaging, PET scanning was performed with an identical axial FOV for 2 to 4 min/table position, depending on condition of the patient and scanner performance. An iterative algorithm with CT scan-derived attenuation correction was used to reconstruct all PET images with a 50-cm FOV. We used an anthropomorphic body phantom (NEMA PET Sensitivity Phantom [NU2-2001]; Data Spectrum Corporation) to minimize variations in SUV among the institutions.[15] To decrease interinstitution SUV inconsistencies, a calibration factor was determined by dividing the actual SUV by the gauged mean SUV in the phantom background. The final SUV used in this study was referred to as the revised SUVmax.[16,17] The original SUVmax values were determined by radiologists from each institution.

Follow-up Evaluations

All patients who underwent lung resection were followed up from their day of surgery. For the first 2 years, postoperative follow-up procedures included a physical examination and chest roentgenogram every 3 months and chest and abdominal CT scan examinations every 6 months. Subsequently, a physical examination and chest roentgenogram were performed every 6 months, and a chest CT scan examination was performed each year.

Statistical Analysis

Results are presented as counts and percentages or as medians, unless stated otherwise. A χ^2 test was used to compare categorical variable frequencies. Fisher exact test was used when sample sizes were small. Recurrence-free survival (RFS) was defined as the time from the date of surgery until the first event (relapse or death from any cause) or the last follow-up. Overall survival (OS) was defined as the time from the date of surgery until death from any cause or the last follow-up. The Kaplan-Meier method was used to assess RFS and OS durations, and these were compared by log-rank test. To assess the potential independent effects of the surgical procedure on RFS, we used multivariate analyses with a Cox proportional hazards model. SPSS, version 10.5 (IBM Corporation) software was used for statistical analysis. The level of significance was set at $P < .05$.

Results

Table 1 shows the characteristics of patients with GGO-dominant tumors. Two hundred thirty-nine of 610 patients (39.2%) had GGO-dominant tumors that had a >50% GGO component. No 30-day postoperative mortality was observed for this population. The median follow-up period after surgery was 42.2 months. Patients with GGO-dominant tumors rarely had pathologically invasive tumors and lymph node metastases. Table 2 shows the distribution of operative procedures for each tumor size (clinical T1a and T1b). Sublobar resections, such as wedge resection and segmentectomy, were more likely performed in T1a tumors, whereas lobectomy was more likely performed in T1b tumors.

Recurrences developed in two patients with GGO-dominant tumors during the follow-up period (Table 3). One patient was an 82-year-old man with a 1.0-cm solid tumor size and with an SUVmax of 1.5 T1b (2.6 cm); peritoneal recurrence developed in this patient 23 months after left-sided S6 segmentectomy. The other patient was a 61-year-old woman with a 1.2-cm solid tumor size and a tumor SUVmax of 1.8 T1b (3.0 cm); brain metastasis developed in this patient 24 months after right-sided middle lobectomy.

Table 1—*Clinicopathologic Features of Patients With GGO-Dominant Tumors*

Variable	GGO-Dominant Tumors (n = 239)
Age, y	65 (31-89)
Male sex	94 (39.3)
Whole tumor size, cm	1.8 (0.7-3.0)
Solid tumor size, cm	0.2 (0-1.2)
SUVmax	0.9 (0-9.8)
Clinical T descriptor	
1a	155 (64.9)
1b	84 (35.1)
Procedure	
Lobectomy	90 (37.7)
Segmentectomy	56 (23.4)
Wedge resection	93 (38.9)
Positive invasion	
Lymphatic	3 (1.3)
Vascular	2 (0.8)
Pleural	1 (0.4)
Positive lymph node metastasis	2 (0.8)

Data are presented as median (range) or No. (%). GGO = ground glass opacity; SUVmax = maximum standardized uptake value.

Table 2—*Distribution of Operative Procedures in Patients With GGO-Dominant Clinical T1a and T1b Lung Adenocarcinoma*

Procedure	T1a Tumor (n = 155)	T1b Tumor (n = 84)	*P* Value
Wedge resection	79 (50.9)	14 (16.7)	...
Segmentectomy	37 (23.9)	19 (22.6)	<.001
Lobectomy	39 (25.2)	51 (60.7)	...

Data are presented as No. (%). See Table 1 legend for expansion of abbreviation.

There was no significant difference in 3-year RFS among patients with GGO-dominant tumors who underwent lobectomy (96.4%), segmentectomy (96.1%), and wedge resection (98.7%, $P = .44$) (Fig 1A). Three-year OS also was not significantly different among patients with GGO-dominant tumors who underwent lobectomy (97.6%), segmentectomy (98.2%), and wedge resection (98.7%, $P = .66$) (Fig 1B).

There was no difference in pathologic invasiveness, including lymphatic, vascular, or pleural, between patients with T1a GGO-dominant tumors and those with T1b tumors (Table 4). For patients with T1b GGO-dominant tumors, there was no significant difference in 3-year RFS among those who underwent lobectomy (93.7%), segmentectomy (92.9%), and wedge resection (100%, $P = .66$) (Fig 1C). Likewise, there was no difference in 3-year OS among patients with T1b GGO-dominant tumors who underwent lobectomy (95.9%), segmentectomy (100%), and wedge resection (100%, $P = .56$) (Fig 1D).

A multivariate Cox proportional hazards model for RFS included the preoperative variables of age, sex, clinical T descriptor, solid tumor size, SUVmax, and surgical procedure. However, none of these variables were independent prognostic factors (Table 5).

Discussion

The results of this study showed that patients with GGO-dominant clinical stage IA lung adenocarcinomas rarely had pathologically invasive tumors and had an excellent prognosis. These findings were consistent with previous reports showing that GGO-dominant lung adenocarcinoma had low malignant potential and good prognosis.[6,7] In addition, the current study showed that 3-year RFS and OS after sublobar resection were similar to those after lobectomy, without significant differences in GGO-dominant clinical stage IA lung adenocarcinoma.

Table 3—*Recurrences in Patients With GGO-Dominant Tumors*

Patient	Age, y	Sex	Whole Tumor Size, cm	Solid Tumor Size, cm	SUVmax	Procedure	ly	v	pl	n	Recurrence Site	Outcome
1	82	M	2.6	1.0	1.5	Segmentectomy	0	0	0	0	Peritoneum	25 mo, alive
2	61	F	3.0	1.2	1.8	Lobectomy	0	0	0	0	Brain	67 mo, alive

F = female; ly = lymphatic invasion; M = male; n = lymph node metastasis; pl = pleural invasion; v = vascular invasion. See Table 1 legend for expansion of other abbreviations.

Sublobar resection generally is indicated for a small lung cancer, such as those ≤2 cm.[3,18,19] However, in the current study, GGO-dominant T1b tumors rarely showed pathologic invasiveness or lymph node metastasis. Moreover, there were no differences in 3-year RFS and OS between patients with GGO-dominant

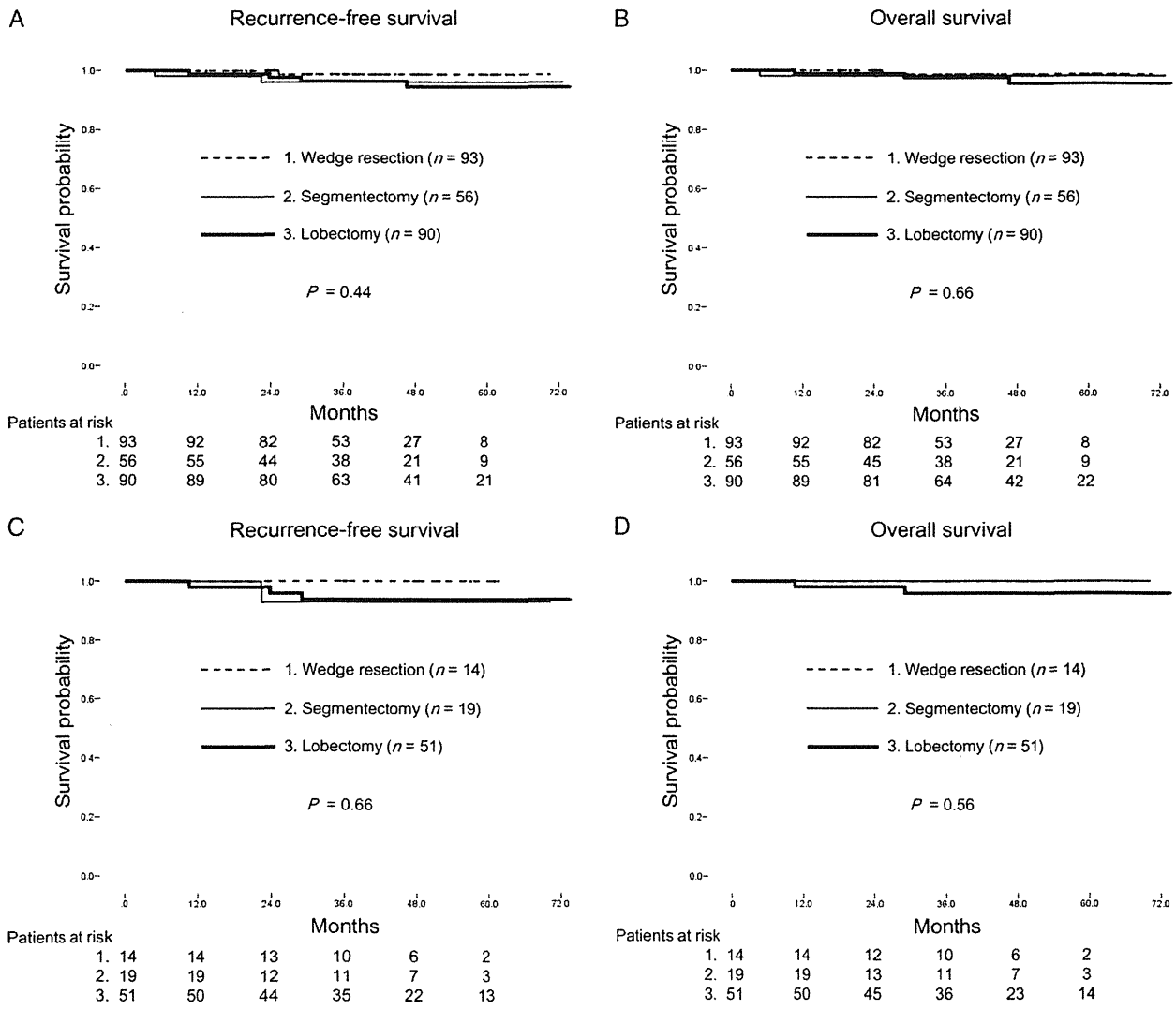

FIGURE 1. Recurrence-free survival (RFS) and overall survival (OS) curves for patients with ground-glass opacity (GGO) tumors who underwent lobectomy and sublobar resection. A, Three-year RFS rate for patients with GGO-dominant tumors who underwent wedge resection (98.7%; mean RFS, 69.8 mo; 95% CI, 68.6-70.9 mo), segmentectomy (96.1%; mean RFS, 70.3 mo; 95% CI, 67.3-73.4 mo), and lobectomy (96.4%; mean RFS, 71.4 mo; 95% CI, 61.9-73.7 mo; $P = .44$). B, Three-year OS rate for patients with GGO-dominant tumors who underwent wedge resection (98.7%; mean OS, 69.8 mo; 95% CI, 68.6-70.6 mo), segmentectomy (98.2%; mean OS, 71.4 mo; 95% CI, 69.0-73.7 mo), and lobectomy (97.6%; mean OS, 72.0 mo; 95% CI, 70.0-74.0 mo; $P = .66$). C, Three-year RFS rate for patients with GGO-dominant T1b tumors who underwent wedge resection (100%; mean RFS, not determined), segmentectomy (92.9%; mean RFS, 66.7 mo; 95% CI, 60.3-73.1 mo), and lobectomy (93.7%; mean RFS, 70.3 mo; 95% CI, 66.7-73.9 mo; $P = .66$). D, Three-year OS rate for patients with GGO-dominant T1b tumors who underwent wedge resection (100%; mean OS, not determined), segmentectomy (100%; mean OS, not determined), and lobectomy (95.9%; mean OS, 71.3 mo; 95% CI, 68.3-74.3 mo; $P = .56$).

Table 4—*Pathologic Findings for GGO-Dominant T1a and T1b Tumors*

Variable	T1a Tumors (n = 155)	T1b Tumors (n = 84)	*P* Value
Lymphatic invasion	1 (0.6)	2 (2.4)	.28
Vascular invasion	1 (0.6)	1 (1.2)	1.0
Pleural invasion	0 (0)	1 (1.2)	.35
Lymph node metastasis	0 (0)	2 (2.4)	.12

Data are presented as No. (%). See Table 1 legend for expansion of abbreviation.

T1b tumors who underwent lobectomy and those who underwent sublobar resection. Therefore, GGO-dominant T1b tumors could also be candidates for sublobar resection. We recommend segmentectomy and not wedge resection for sublobar resection of a GGO-dominant T1b tumor because these tumors could involve lymph node metastasis, and taking a sufficient surgical margin by wedge resection often is difficult in a T1b tumor.

In the current study, we found that two of 84 patients (2.4%) with GGO-dominant T1b tumors had lymph node metastases. No lymph node metastases were found for those with GGO-dominant T1a tumors. However, segmentectomy can approach hilar lymph nodes, whereas wedge resection cannot; thus, we should choose an optimal surgical procedure to avoid local recurrence in hilar lymph nodes, surgical stump, or residual lung. Segmentectomy would be superior to wedge resection for taking a sufficient surgical margin and for assessing hilar lymph nodes. Because sublobar resection includes both wedge resection and segmentectomy, it is necessary to distinguish between wedge resection and segmentectomy to clarify which procedure was used.

We encountered two distant recurrences with GGO-dominant T1b tumors: a brain metastasis after lobectomy and a peritoneal metastasis after segmentectomy, which could not be avoided even by standard lobectomy. One of the most important issues with sublobar resection is local control. Sublobar resection would be suitable for a GGO-dominant tumor because in this study, no intrathoracic local recurrence was observed, although a longer follow-up will be necessary before arriving at a definitive conclusion because of the indolent nature of GGO-dominant tumors.

Table 5—*Multivariate Analysis for Recurrence-Free Survival for Patients With GGO-Dominant Tumors*

Variable	HR (95% CI)	*P* Value
Age	1.08 (0.97-1.20)	.15
Male vs female sex	0.85 (0.18-3.91)	.83
T1b vs T1a descriptor	1.17 (0.20-6.70)	.86
Solid tumor size	6.37 (0.45-89.9)	.17
SUVmax	0.99 (0.52-1.90)	.99
Lobectomy vs sublobar resection	1.27 (0.20-7.93)	.82

HR = hazard ratio. See Table 1 legend for expansion of other abbreviations.

In the current study, the surgical procedure used and T descriptors were not independent prognostic factors of RFS in patients with GGO-dominant tumors, which also supports that a sublobar resection, such as a wedge resection or a segmentectomy, is suitable for GGO-dominant clinical stage IA lung adenocarcinomas, even for T1b tumors. In addition, solid tumor size and SUVmax were not independent prognostic factors of RFS. We previously reported that solid tumor size on HRCT scan and SUVmax on FDG-PET/CT scan were independent prognostic factors for lung adenocarcinoma.[14,20-22] However, patients with GGO-dominant lung adenocarcinomas have an excellent prognosis regardless of solid tumor size or SUVmax.

We speculate that GGO-dominant tumors indicate a uniform group exhibiting less tumor invasiveness and a favorable prognosis. In the current study, GGO-dominant tumors had small solid tumor sizes (median, 0.2 cm) and low SUVmax (median, 0.9). Prognosis based on solid tumor size and SUVmax may be useful, particularly for solid-dominant lung adenocarcinomas. In a previous study, we proposed N0 criteria that use a solid tumor size of < 0.8 cm or SUVmax of < 1.5 for predicting true N0 in clinical stage IA lung adenocarcinoma; patients who met these N0 criteria could be candidates for sublobar resection, such as wedge resection and segmentectomy.[11] Furthermore, patients with GGO-dominant tumors as well as those who meet the N0 criteria can be good candidates for wedge resection or segmentectomy.

Because this was a retrospective study, it is possible that patients who underwent sublobar resection were highly selective. Clinical trials comparing surgical results between lobectomy and sublobar resection (segmentectomy or wedge resection) for clinical T1aN0M0 NSCLC are currently being conducted by the Cancer and Leukemia Group B (CALGB 140503) and the Japan Clinical Oncology Group/West Japan Oncology Group (JCOG0802/WJOG4607L). These study results should indicate the significance of sublobar resection for small NSCLCs.[23] Regarding T1b tumors, a prospective study of segmentectomy for GGO-dominant tumors is warranted.

In conclusion, GGO-dominant clinical stage IA lung adenocarcinomas are a uniform group of tumors that exhibit low-grade malignancy and have a favorable prognosis. Patients with GGO-dominant tumors can be treated with wedge resection for T1a tumors and segmentectomy for T1b tumors.

ACKNOWLEDGMENTS

Author contributions: Drs Tsutani and Okada had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Dr Tsutani: contributed to study design, data acquisition, manuscript preparation, and approval of the final manuscript.
Dr Miyata: contributed to manuscript preparation and approval of the final manuscript.
Dr Nakayama: contributed to data acquisition, manuscript preparation, and approval of the final manuscript.
Dr Okumura: contributed to manuscript preparation and approval of the final manuscript.
Dr Adachi: contributed to manuscript preparation and approval of the final manuscript.
Dr Yoshimura: contributed to data acquisition, manuscript preparation, and approval of the final manuscript.
Dr Okada: contributed to study design, manuscript preparation, and approval of the final manuscript.
Financial/nonfinancial disclosures: The authors have reported to *CHEST* that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

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(肺癌. 2014;54:173-179)

ORIGINAL ARTICLE

自施設で施行した肺癌 ALK 検査（IHC と FISH）の院外検査との比較検討

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Comparison of In-house and Outsourced ALK Tests (IHC and FISH) in Patients with Lung Cancer

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ABSTRACT —— ***Objective.*** In the treatment of advanced ALK-positive lung cancer, it is important to establish a robust system for ALK testing that is both accurate and rapid. In this study, we compared the duration of examination and rate of concordance between an in-house and outsourced ALK test. ***Methods.*** We performed in-house ALK tests of 43 specimens (including samples for immunohistochemistry (IHC) and fluorescene *in situ* hybridization (FISH)) on which outsourced ALK tests had been conducted between May 2012 and May 2013 and compared the results of the two tests. The EnVision FLEX+ method and iAEP method were used for outsourced and in-house IHC, respectively. In cases in which IHC was determined to be positive, FISH was conducted using the split assay method at each institution. ***Results.*** The specimens under investigation included 20 operative specimens, 20 biopsy specimens and three cytological examination specimens. The average duration of examination was 6.2 and 12.3 days for the outsourced IHC and FISH analyses and 3.0 and 8.0 days for the in-house IHC and FISH analyses, respectively. Among the cases in which both outsourced and in-house IHC was performed, the positive and negative conformity rates for the in-house IHC versus the outsourced IHC were 100% and 86.5%, respectively. The results of IHC were discordant in five cases, all of which included operative specimens that were negative on outsourced IHC and positive on in-house IHC. One of these five cases was also positive on FISH. The findings of the in-house and outsourced FISH analyses were concordant in all cases. ***Conclusions.*** The in-house ALK test was associated with a shortened duration of examination compared with that observed for the outsourced ALK test, with a high rate of conformity between the two assays. However, there were several discordant cases on IHC due to differences in the detection reagents used at each institution. In addition, an iScore value of 1 or higher was classified as ALK-positive on the in-house IHC assay, and the technicians may have reviewed different regions in the same operative specimen.

(*JJLC.* 2014;54:173-179)

KEY WORDS —— Lung cancer, ALK, FISH, IHC, iAEP method

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Received February 23, 2014; accepted June 30, 2014.

要旨 —— **目的.** 進行 ALK 肺癌治療には正確かつ迅速な ALK 検査体制を要する．院内 ALK 検査導入に際し外部検査受託機関（院外）と院内の ALK 検査精度と判定期間を比較検討した．**方法.** 2012/5～2013/5 に院外 ALK 検査（IHC・FISH）を施行した 43 例に対し院内 ALK 検査を行った．院外 IHC は EnVision FLEX＋法，院内 IHC は iAEP 法を用い，陽性例に各施設で FISH（split assay 法）を行った．**結果.** 手術検体は 20 例，結果判定

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受付日：2014 年 2 月 23 日，採択日：2014 年 6 月 30 日.

期間は院外IHC/FISH 6.2/12.3日，院内IHC/FISH 3.0/8.0日．院外・院内IHCの陽性/陰性一致率は100/86.5%，IHC不一致例(5例)は全て手術検体で院外陰性・院内陽性，うち1例はFISH陽性であった．一方FISHは全例一致した．**結論.** 院内ALK検査は判定期間を短縮した．院内・院外の検査一致率は高いが，IHC不一致を認めた．理由としては施設間で使用する検出試薬が違うこと，院内ではiScore 1以上を陽性と判定したこと，施設間で同一手術標本の判定部位が異なる可能性などが考えられた．

索引用語 —— 肺癌，ALK，FISH，IHC，iAEP法

目 的

*EML4-ALK*融合遺伝子陽性肺癌は，2007年にSodaらにより初めて同定された.[1] *EML4-ALK*融合遺伝子は非小細胞肺癌の2〜6%に認められ,[1,2] ALK蛋白のチロシンキナーゼドメインの持続的な発現を引き起こし，腫瘍増殖に関与している.[3] CrizotinibはALKチロシンキナーゼを阻害する薬剤であり，ALK肺癌に対して用いられている.[4,5] 進行ALK肺癌に対する一次治療においてcrizotinibと化学療法（pemetrexedおよびプラチナ製剤）を比較した第III相試験（PROFILE 1014）では，crizotinib群がprogression-free survival（PFS）10.9ヶ月，overall response rate（ORR）74%，化学療法群がPFS 7.0ヶ月（ハザード比0.454，$p<0.0001$），ORR 45%（$p<0.0001$）であり，crizotinibはALK肺癌に対し高い治療効果があることが示された．またPROFILE 1007では，プラチナ製剤を含む治療歴がある症例を対象として，2次治療でcrizotinibと標準化学療法との比較が行われた.[6] その結果crizotinib群のPFSは7.7ヶ月で，化学療法群の3.0ヶ月よりも有意にPFSを延長した（ハザード比0.49，$p<0.001$）．

ALK肺癌へのALK阻害剤の投与機会を逸しないためには正確かつ迅速な診断を要する．Crizotinibはfluorescence *in situ* hybridization（FISH）により同定された進行ALK陽性非小細胞肺癌に対し，United States Food and Drug Administration（FDA）で承認されている.[7] 現在のALK肺癌の診断アルゴリズムは，immunohistochemistry（IHC）陽性例に対しFISHを行うのが標準である.[1]

当院ではALK検査（IHCおよびFISH）を外部検査受託機関（院外）で行ってきた．しかしALK検査を院外で行うよりも自施設で行った方が検査結果までの期間が短縮される利点があると考え，IHCを神奈川県立がんセンター病理診断科で，またFISHを神奈川県立がんセンター検査科で施行し，ALK検査体制を立ち上げた．今回自施設でALK検査を導入するにあたり，院内・院外のIHC, FISHの検査判定期間およびその検査精度について比較・検討を行った．

方 法

2012年5月〜2013年5月の期間に提出された*EGFR*遺伝子変異が陰性である原発性肺癌の手術検体・生検検体・細胞診検体のうち，院外でALK検査（IHCおよびFISH）が施行された43例を対象とした．自施設で再度ALK検査を施行し，院外ALK検査との一致率を算出した．

院内・院外IHCではともに4 μmのformalin fixed paraffin embedded（FFPE）組織切片を用い，一次抗体として5A4モノクローナル抗体を用いた．院内IHCではintercalated antibody-enhanced polymer（iAEP）法（Nichirei Biosciences Inc., Tokyo, Japan）を，院外IHCではEnVision FLEX＋法（Dako Denmark A/S, Denmark）を用いてIHCを施行した．評価法は院内IHCではiScoreを用いて4段階（0＝陽性腫瘍細胞なし，1＝50%≧陽性腫瘍細胞率>0%，2＝80%≧陽性腫瘍細胞率>50%，3＝陽性腫瘍細胞率>80%）で評価し,[8,9] iScore 1以上をALK陽性と判定した．院外IHCでは陽性腫瘍細胞率＝0%か，または陽性腫瘍細胞率>0%かの2段階評価で行い，後者をALK陽性と判定した．

院内および院外のいずれにおいても，IHC陽性例に対し4 μmのFFPE組織切片を用いて，split assay法によるFISH（Vysis LSI ALK（2p23）Dual Color, Break Apart Rearrangement Probe；Abbott Molecular Inc., Chicago, IL, USA）を行った．3'側（赤色）と5'側（緑色）がシグナル2個分以上離解した場合を"split signalあり"と判定した．FISH陽性率（%）は20個の腫瘍細胞中に占めるsplit signalを有する腫瘍細胞または単一の赤色signalを有する腫瘍細胞の割合を示し，陽性率15%以上をALK陽性と判定した．

ALK結果の判定期間とは，院外では検査依頼日から検査結果が当院に届くまでの期間とした．一方院内ではALK検査依頼日から病理診断科でIHCの結果報告が可能となった日，もしくはFISHの結果が神奈川県立がんセンター検査科より病理診断科に届くまでの期間とした．

結　果

Table 1 に ALK 検査を行った 43 例の患者背景を示した．検体材料は手術検体 20 例，生検検体 20 例，細胞診検体 3 例であった．年齢中央値は 65（38～82）歳で男性は 26 例（60.5%），腺癌は 37 例（86.0%）であった．院外 IHC の結果判定期間は平均 6.2（4～12）日，IHC と FISH の合計判定期間は平均 12.3（9～16）日であった．院内 IHC の結果判定期間は平均 3.0（1～8）日，IHC と FISH の合計判定期間は約 8.0 日であった．

ALK 検査を施行した 43 例の内訳を Figure 1 に記した．院内・院外 IHC ともに陽性であった 6 例に対し，院内・院外 FISH を施行した．院内 IHC 陽性，院外 IHC 陰性であった 5 例中，検体が十分量なかった 1 例を除いた 4 例に対して院内 FISH を施行した．

Table 1. Patient Characteristics Among the Cases in Which ALK Tests Were Performed

N = 43	
Median age (years)	65 (38-82)
Male (%)	26 (60.5)
Histopathological diagnosis	
Adenocarcinoma	37
LCNEC	1
Pleomorphic carcinoma	1
SCLC	1
NSCLC	3
Samples under examination	
Operative specimen	20
Biopsy specimen	20
Cytology specimen	3

LCNEC, large-cell neuroendocrine carcinoma; SCLC, small-cell lung carcinoma; NSCLC, non-small cell lung carcinoma.

Table 2 には院内・院外 IHC を施行した 43 例の結果を示した．院外 IHC 陽性 6 例は全て院内 IHC 陽性であった（陽性一致率 100%）．また院外 IHC 陰性 37 例中 32 例は院内 IHC が陰性であった（陰性一致率 86.5%）．不一致は 5 例（11.6%）認め，全例院外 IHC 陰性，院内 IHC 陽性であり，いずれも手術検体であった．

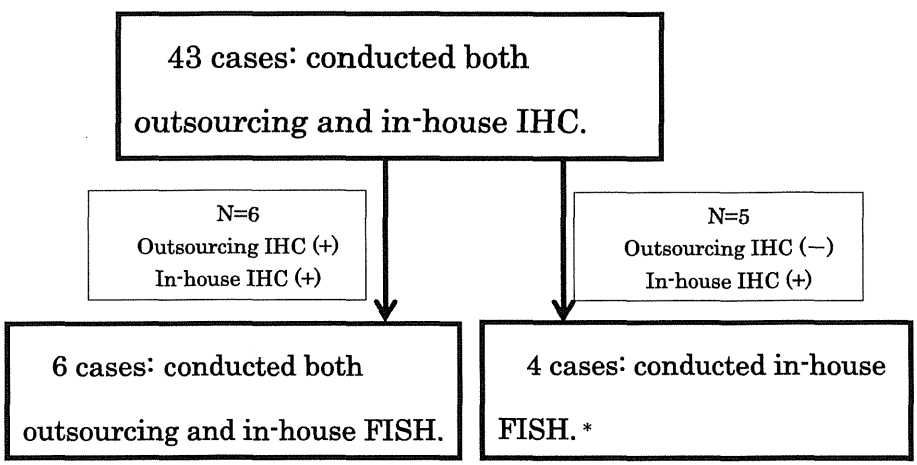
Table 3 には院内 IHC 陽性であった 11 例中，検体が微量のため FISH が施行できなかった 1 例を除いた 10 例の iScore，FISH の結果，および院外 IHC の結果を示した．iScore 3，2，1 であった症例はそれぞれ 4 例，3 例，3 例認め，そのうち FISH 陽性は 4 例（100%），2 例（66.7%），1 例（33.3%），院外 IHC 陽性は 4 例（100%），2 例（66.7%），0 例（0%）であった．

Table 4 には 5 例の不一致例（院外 IHC 陰性，院内 IHC 陽性）の iScore および院内 FISH の結果を示した．不一致例はいずれも iScore は 1 点または 2 点であったが，5 例中 1 例（Case 4）において院内 FISH が陽性であった．本症例（Case 4）の IHC および FISH の結果を Figure 2 に示した．IHC では iScore 1 であったため陽性と判定し，FISH では split signal を全体の 17%（赤丸部）認めたため FISH 陽性と判定した．

Table 5 では IHC 陽性例に対し院内・院外 FISH を行った 6 例の結果を示した．院内・院外 FISH はいずれも陽性で全て一致した（陽性一致率 100%）．

結　論

自施設で ALK 検査を施行することで院外よりも結果



* FISH was not conducted in one case due to the small sample.

Figure 1. Breakdown of the patients who underwent in-house and outsourced ALK tests.

判定期間を短縮することができた．その理由の1つとして，院内検査は検査開始日の設定がないことが挙げられる．すなわち院内検査では被検体が病理部または検査科に到着した日より検査が直ちに開始できる体制ができている．一方院外検査では被検体の到着日より検査が開始されるわけではないため，その分結果判定期間の遅延が生じると考えられた．また院外へ被検体を搬送するのに要する時間も，結果判定の遅延の原因と考えられた．

本検討より院内IHCと院外IHCとの陽性一致率，陰性一致率は高く，院内IHCをALK肺癌検出のスクリーニング法として用いることは妥当と考えられた．しかし一方で院外IHC陰性，院内IHC陽性となる不一致例が5例(11.6%)生じた．この5例は全て手術検体であり，うち1例は院内FISHが陽性であった(Table 4, Figure 2)．院内・院外ALK-IHC判定の不一致が生じる理由としては以下の3つが考えられた．①IHCに用いるALK検出試薬(iAEP法とEnVision FLEX＋法)が施設間で違うこと，②院内IHCではiScore 1以上をALK陽性と判定していること，③施設間で同一手術標本の判定部位が異なる可能性．

IHCで用いられるiAEP法とEnVision FLEX＋法はいずれも免疫組織化学酵素抗体法を基礎とした手法である．iAEP法は抗ALK(clone：5A4)マウス・モノクローナル抗体を反応させ，ブリッジ試薬であるintercalating reagentを一次抗体とジアミノベンジジン（DAB）ポリマー試薬との間に介在させることによりALK融合蛋白の染色感度および検出感度を上げる方法である．[8] 一方EnVision FLEX＋法ではiAEP法と同様に一次抗体として抗ALK（clone：5A4）マウス・モノクローナル抗体を

Table 2. Results of the In-house and Outsourced IHC Assays

	In-house IHC positive	In-house IHC negative	Total
Outsourcing IHC positive	6*	0	6
Outsourcing IHC negative	5†	32*	37
Total	11	32	43

*Among the 43 cases in which both outsourced and in-house IHC was conducted, the positive and negative conformity rates for in-house IHC versus outsourced IHC were 100% (6/6) and 86.5% (32/37), respectively, with five cases in which the results of IHC were discordant.

†All five cases of discordant results included operative specimens.

IHC, immunohistochemistry.

Table 3. Results of the In-house FISH Analyses for Each iScore Among the In-house IHC-positive Cases

iScore of in-house IHC positive cases	N (total 10)	In-house FISH positive cases (%)	Outsourcing IHC positive cases (%)
iScore 1	3	1 (33.3)	0 (0)
iScore 2	3	2 (66.7)	2 (66.7)
iScore 3	4	4 (100)	4 (100)

IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization.

Table 4. iScore and FISH Results in the Five Cases with Discordant In-house and Outsourced IHC Findings

Case	Specimen	Outsourcing IHC	In-house IHC		In-house FISH	
			(+/−)	iScore	(+/−)	Positive rate (%)
1	operative	(−)	(+)	1	could not evaluate*	
2	operative	(−)	(+)	1	(−)	8
3	operative	(−)	(+)	2	(−)	6
4†	operative	(−)	(+)	1	(+)	17
5	operative	(−)	(+)	1	(−)	6

*FISH was not conducted in Case 1 due to the small sample.

†All five cases of discordant findings included operative specimens that were negative on outsourced IHC and positive on in-house IHC, with one cases (Case 4) that was positive on in-house FISH.

IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization.

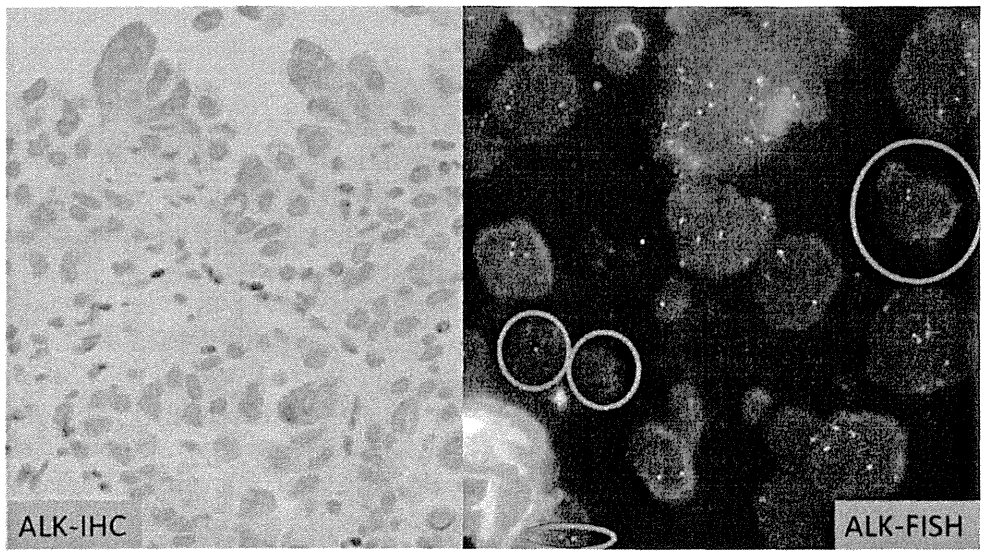


Figure 2. Among the five cases with discordant IHC findings (all of which were negative on outsourced IHC and positive on in-house IHC), one case (Case 4) was found to be FISH-positive. The sample was considered to be IHC-positive (iScore 1+) because 37% of the tumor cells exhibited a cytoplasmic reaction (left) and FISH-positive because 17% of the tumor cells demonstrated splitting of red and green signals (red circle) (right).

Table 5. Results of the In-house and Outsourced FISH Assays

	In-house FISH positive	In-house FISH negative	Total
Outsourcing FISH positive	6*	0	6
Outsourcing FISH negative	0	0	0
Total	6	0	6

*The results of the in-house and outsourced FISH analyses were concordant in all six cases.

FISH, fluorescence *in situ* hybridization.

反応させる．そして一次抗体と西洋ワサビペルオキシダーゼを多数標識した標識ポリマーにマウスリンカー試薬を反応させ，ペルオキシダーゼの酸化作用でDABを発色させてALK蛋白を検出する．一般的に肺癌のALK発現は，ALK再編成を有するanaplastic lymphomaと比較して低いため，[10] iAEP法，EnVision FLEX＋法を用いた増感法を要する．両者の原理は類似しているが，各手法の一次抗体と高分子ポリマーとを介在させる試薬およびキット内の各試薬の濃度などが異なる．

IHCで用いられる一次抗体には，ALK1，5A4，D5F3，SP8などがあり，そのうちALK1と5A4抗体とreverse transcription-polymerase chain reaction（RT-PCR）およびFISHとの間には良好な相関関係が示されている．[11-18] しかしこれらの一次抗体間でその相関性の違いを比較検討した報告は少ない．[8,15] 過去の報告によるとD5F3抗体とALK1抗体とを比較し，前者がよりRT-PCRやFISHの結果との相関性が高かった．[15] またTakeuchiらは5A4のマウス・モノクローナル抗体を用いてiAEP法を行うことで，他の一次抗体と比較してRT-PCRの結果と相関性が高かったと報告している．[8] 5A4抗体とFISHとの相関性については，Paikらは5A4抗体によるIHCの感度は100%，特異度は95.8%と報告し，[16] またPaikらは別の報告において感度は100%，特異度は96.2%と報告している．[19] iAEP法とEnVision FLEX＋法はいずれもFISHと相関性が高く，ALK蛋白の検出感度が高いとされているが，両者を直接比較検討した論文は，我々が知る限りはない．現在FISHとIHCとの不一致例がALK阻害剤による肺癌治療で問題になっているが，増感法の差が不一致を生じさせている可能性もあるため，両検査法でのFISHの相関性を，同一症例群を用いて検討すべきと考えられた．

IHC不一致の2つ目の理由は，院内IHCではiScore 1以上をALK陽性と判定していることである．現時点では複数のIHCの評価法および判定基準が存在し，[1,7,9,17,18] 定まった基準がない．iSocreを用いる場合，iScore 3を陽性とすることでFISHの結果と完全に一致するとされている．[1,9] 本検討ではiScore 3の症例は院外IHCと完全

に一致したが，iScore 1，2の症例は院外IHCと完全には一致しなかった(Table 3)．しかし院内IHC陽性(iScore 1)，院外IHC陰性，院内FISH陽性となるALK陽性肺癌(Table 4, Case 4)が検出されたことから，iScore 1であってもFISHを確認する意義はあると考えられる．IHC弱～中等度陽性症例においてFISHとの不一致が問題となっているが，[20] iAEP法においてiScore 1以上をALK陽性と判定することがEnVision FLEX＋法による判定結果と不一致が生じる要因の1つになっている可能性がある．それぞれの増感法において，明確なIHC判定基準を設けることが必要と考えられた．

3つ目の理由としては，同一の手術標本であっても，施設ごとで判定者によって判定する部位が異なる可能性が挙げられる．本検討では手術検体材料でのみ不一致が生じた．生検検体や細胞診検体では検体量が少ないため観察範囲が狭いが，手術検体では観察範囲が広いため，判定者により判定部位およびその評価がばらつく可能性がある．FISHとの相関性を高めるためには，手術検体材料でのIHCの評価が他の検体材料と同じ測定法・評価法で良いかを再検討する必要があると考えられた．

しかしこれらの原因によって生じるIHC間不一致の症例が，実際にALK阻害剤に奏効するのかが本質的な問題と考えられる．2013年5月27日に日本肺癌学会バイオマーカー委員会より提起された「FISH法と高感度IHC法の不一致についてのお知らせと対応」において，不一致は2.1%（48/2337例）に認められ，不一致症例はcrizotinibへの奏効性が低い可能性が示唆された．今回，院内・院外IHC不一致例(5例)に対してcrizotinibは使用していないため評価はできなかった．各増感法および各判定基準によるIHCの結果が，ALK阻害剤の奏効性にどのように影響するかを検討する必要があると考えられた．

今回用いたVysis break-apart FISH probe kitは高感度・高特異度なALK rearrangement検出法であり，FDAにより現在承認されている．[7] 本検討ではFISH判定は院内・院外で完全に一致し（Table 5)，院内FISHは再現性の高いALK肺癌検出法であることが示された．しかし今回院内IHC陽性・院外IHC陰性となった5例中，院内FISH陰性を3例認めた(Table 4)．院内IHCと院内FISHの不一致の理由としては全長ALKを陽性とした可能性，検体の質の問題，腫瘍細胞が検体中に含まれていなかった可能性などが考えられる．[9] 今後はこのような症例に対し再度FISHを行うか，またはRT-PCRで確認する必要があると考えられた．

本検討より院内ALK検査はALK判定までの期間を短縮できたことが示された．また院内・院外ALK検査(IHC，FISH)の一致率は高く，院内でALK検査は施行可能と考えられた．今後PROFILE1014の結果を受けてcrizotinibがALK肺癌の一次治療として用いられる可能性があり，院内で短期間に精度の高いALK検査が達成できることは，ALK肺癌の臨床治療選択において利点となりうることが考えられる．しかし院内IHCと院外IHCの間に一部不一致例を認めた．この不一致の理由を今後さらに症例集積した上で検討すると同時に，不一致例のALK阻害剤の奏効性を評価することが必要不可欠と考えられた．

本論文内容に関連する著者の利益相反：なし

謝辞：本論文作成にあたり，札幌医科大学医学部附属フロンティア医学研究所の佐久間裕司先生，および神奈川県立がんセンター検査科の関口博信氏よりご協力をいただきましたことを，心より感謝申し上げます．

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