Table 2 Correlations between age group and clinicopathological features, including *EGFR* status

Characteristics	No. of patients			
	Total	≥80 years	<80 years	p^a
	(*n* = 388)	(*n* = 29, 7.5%)	(*n* = 359, 92.5%)	
Mean age, yr ± SD[b]	66.6 ± 10.0	82.6 ± 2.41	65.3 ± 9.29	<0.001
Gender				0.246
Male	228	20	208	
Female	160	9	151	
Histology				0.034
Adenocarcinoma	302	18	284	
others	86	11	75	
Biomarker				
EGFR wild type	203	22	181	0.008
EGFR mutation	185	7	178	
KRAS wild type	355	23	332	0.014
KRAS mutation	33	6	27	
Vascular invasion				
Ly -	314	26	288	0.214
Ly +	74	3	71	
V -	261	18	243	0.535
V +	127	11	116	
p-stage				0.080
I	293	18	275	
II / III	95	11	84	
T-factor				0.506
T1	197	13	184	
T2/3	191	16	175	
Tumor diameter (cm)	3.03 ± 1.43	3.00 ± 1.44	3.40 ± 1.24	0.629
N-factor				0.584
N0	322	23	299	
N1/2	66	6	60	
Operation				0.155
Limited resection (wedge/segmentectomy)	80	3	77	
Standard surgery (lobectomy, pneumonectomy)	308	26	282	
Lymph node resection				<0.001
ND0/1/sampling	109	25	156	
ND2	278	4	203	
Smoking				0.024
Non-smoker	157	6	151	
Smoker	231	23	208	
Pre-existing cardiopulmonary comorbidity	203	21	182	0.024

[a]$p < 0.05$ statistically significant.
[b]SD, standard deviation.
EGFR, epidermal growth factor receptor; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; ND, lymph node dissection.

in patients with *EGFR* mutations (100%) than in those with wild-type *EGFR* (66.2%) in the older group (P = 0.226; Figure 1B).

Discussion

In the present study, we first evaluated *EGFR* mutations in resected NSCLC tissue by LH-MSA. LH-MSA is a

Table 3 Region of *EGFR* mutation according to age group

	No. of patients		
EGFR mutations	Total	≥80 years	<80 years
Exon 19	73	3	70
Exon 20	13	0	13
Exon 21	97	4	93
Combined	2	0	2

highly sensitive polymerase chain reaction-based method. Sakuma *et al.* previously evaluated *EGFR* mutations by LH-MSA in our hospital. *EGFR* mutations were detected in 53.2% of NSCLCs and were significantly associated with adenocarcinoma, female sex, and no smoking history [14]. In the present study, we detected EGFR mutations in 47.7% of NSCLCs (Table 1). The presence of an *EGFR* mutation is closely linked to several clinicopathological factors, such as gender, smoking history, and pathological findings. Our results are consistent with those of recent studies reporting that the rate of EGFR mutations is higher among Asians (including Japanese), females, nonsmokers, and adenocarcinomas [14,15]. Although LH-MSA yet has not been generally performed, it is known to be a sensitive and low cost method in scanning the known gene mutation. Furthermore, we can treat many samples in a short time by LH-MSA. Nakajima et al. analyzed *EGFR* mutations using LH-MSA, and confirmed the results by direct sequencing. They concluded that LH-MSA has a high detection capability compared with direct sequencing [16]. Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology indicate that LH-MSA compares favourably with the other method [17].

We then studied the relations between *EGFR* status and clinicopathological factors according to age group (Table 2). Past report suggested the impact of age on *EGFR* mutation, and concluded that age was associated with *EGFR* mutation in lung cancer [18]. In this study, if we analyze the *EGFR* status using the median age of 66 years old as a cutoff, there is no difference between younger and elderly group. Next, we divided the cohort in every ten years old, and we found that the rate of *EGFR* mutation suddenly decreased in a group 80 years or older. Because aging of the population is a global problem, the average life-span older than 80 years old in Japan was worthy of mention to the world. Due to the above reasons, we thought that the age of 80 years old is turning point in consideration of gene profile change, and divided the patients into two groups at 80 years of age. The older group (≥80 years) of patients with NSCLC included significantly higher rates of non-adenocarcinoma, wild-type *EGFR*, *KRAS* mutations, and smokers. There was no difference between the older group and younger group in tumor size, T-factor, or pathological stage. Moreover, in Japan, females outlive males (males 79 years, females 86 years). Of the 29 elderly patients, 9 are females include 7 adenocarcinomas and 4 smokers. *EGFR* mutations were detected in 3 females. The 5-year overall survival rate was 100% regardless of *EGFR* mutation or wild type. When we examined the region of *EGFR* mutation according to age group (Table 3), no exon 20 mutations were found in the older group. Although our study group was small, our results suggest that *EGFR* mutation status might differ

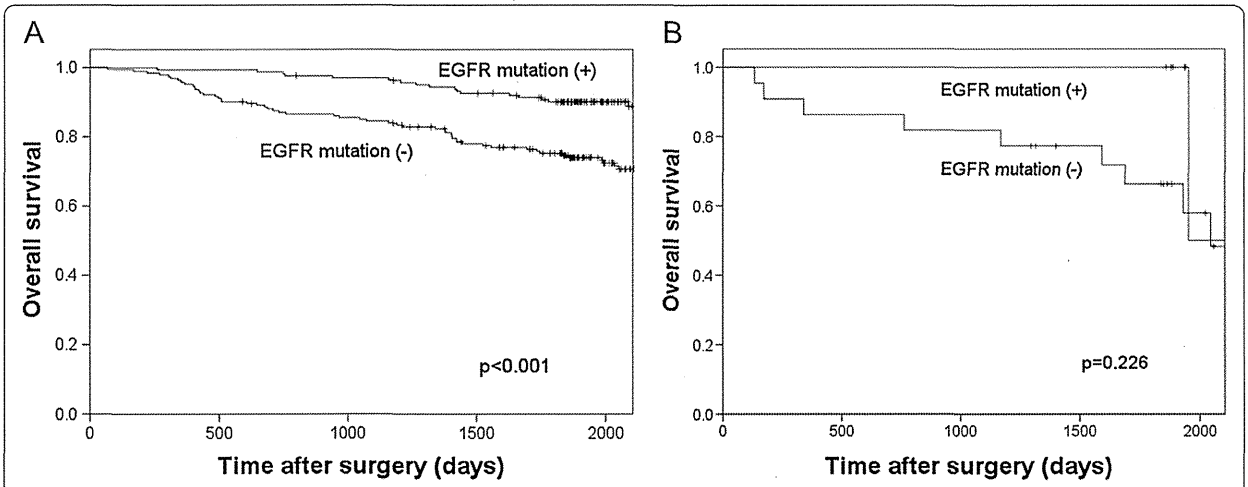

Figure 1 Relations between *EGFR* mutations and outcomes. Kaplan-Meier curve analysis showed that *EGFR* mutation status was significantly associated with survival. **(A)** The 5-year overall survival rate was higher in patients with *EGFR* mutations (90.2%) than in those with wild-type *EGFR* (75.2%) in the younger group ($P < 0.001$). **(B)** In the older group, the 5-year disease free survival rate was 100% among patients with *EGFR* mutations; however, the difference between the two groups was not significant.

between elderly and younger patients with NSCLCs. Given that smoking is one of the causes of the low rate of *EGFR* mutations in the older group, the rate of *EGFR* mutations may increase in the future owing to enlightenment movements such as the WHO Framework Convention on Tobacco Control [19]. Recently, smoking prevalence in Japan is decreasing generally. In particular, the drop of the smoking prevalence in young generation is remarkable. On the other hands, lung cancer mortality in Japan rises, probably it depends on the increase of the lung cancer in an elderly person who had been a smoker [20]. If the low rate of *EGFR* mutations is unrelated to smoking, it is very interesting that EGFR status might be affected by aging. Furthermore, it is reported that the response rate of gefitinib in elderly (aged 70 years or older) patients with advanced *EGFR* mutated NSCLC was 45.5%. EGFR-TKI is more effective than conventional chemotherapy in elderly patients, if we could pay attention to drug discontinuation and dose reduction due to age-related organ dysfunction [21]. On the other hand, NSCLC with exon 20 mutation is resistant for EGFR-TKI. Although our result has no statistical significance due to a small population of elderly patients, the lack of exon 20 mutations might be a characteristic of elderly patients. Large clinical trials are needed to investigate the relation between age group and the response to EGFR-TKI.

Finally, we assessed the relations between the *EGFR* status and outcomes. *EGFR* mutations were associated with significantly better survival than wild-type *EGFR* in the younger group (Figure 1). In the older group, however, the 5-year overall survival rate did not differ significantly according to *EGFR* mutations, and wild-type *EGFR* status and was 100% in patients with *EGFR* mutations. EGFR-TKIs are obviously beneficial in patients with advanced or recurrent NSCLC, but several studies have suggested that *EGFR* mutations might be an independent positive prognostic factor [22]. Our results suggest that elderly patients with NSCLC who have *EGFR* mutations are especially likely to have good outcomes after complete lung resection.

Conclusion

Our results suggest that the *EGFR* status of patients with NSCLC differs according to age group (>80 years vs. ≤80 years). *EGFR* mutation status might be a prognostic marker in elderly patients with completely resected NSCLC.

Additional file

Additional file 1: Table S1. PCR Primers and LH-G Probes Used for Detection of Mutations in *EGFR*.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

Study design: TN, TY, YM and YD; sample collection: TN, HI, TI, KI, Shuji M, TK, HS, FO, KY, MT, and HN; experiments: TN, TY, YM, YD, and Shoichi M; data analysis: TN and TY; preparation of the manuscript: TN, TY, HN, and MM. All authors read and approved the final manuscript.

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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.

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

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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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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．

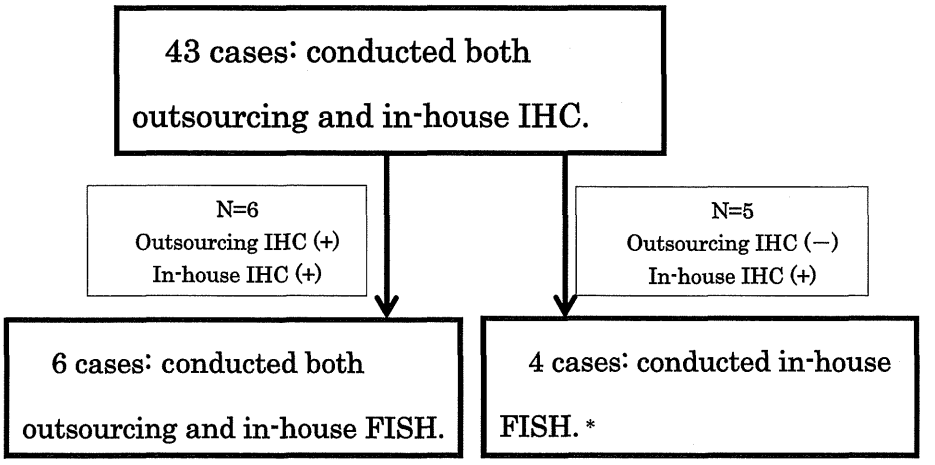
Table 3 には院内 IHC 陽性であった 11 例中，検体が微量のため FISH が施行できなかった 1 例を除いた 10 例の iScore，FISH の結果，および院外 IHC の結果を示した．iScore 3，2，1 であった症例はそれぞれ 4 例，3 例，3 例認め，そのうち FISH 陽性は 4 例（100%），2 例（66.7%），1 例（33.3%），院外 IHC 陽性は 4 例（100%），2 例（66.7%），0 例（0%）であった．

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.

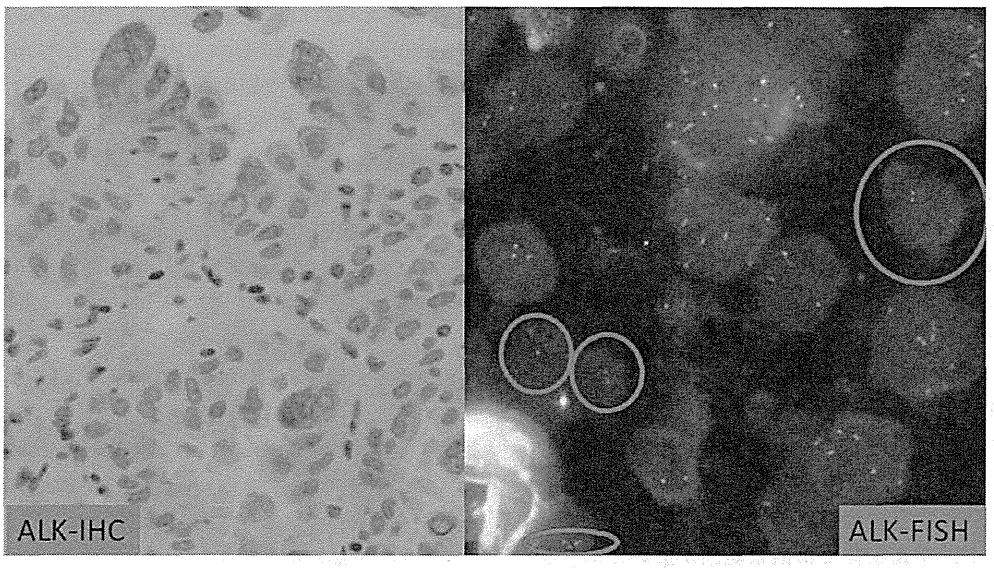


Figure 2. Among the five cases with discordant IHC findings (all of which were negative on outsourced IHC and positive on in-house IHC), one case (Case 4) was found to be FISH-positive. The sample was considered to be IHC-positive (iScore 1+) because 37% of the tumor cells exhibited a cytoplasmic reaction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

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Surgical Treatment for Synchronous Primary Lung Adenocarcinomas

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Background. Surgical treatment has become the mainstay of treatment for multiple primary lung cancers. In particular, the prevalence of synchronous primary lung adenocarcinomas (SPLA) has recently increased, but few studies have evaluated surgical outcomes of patients with SPLA. We reviewed the clinicopathologic features and surgical outcomes of SPLA to identify factors related to survival.

Methods. Data on 2,041 consecutive patients with primary non-small cell carcinoma who underwent surgical resection in our hospital from 1995 through 2009 were retrospectively analyzed.

Results. The SPLA was pathologically diagnosed in 93 patients, including 26 with bilateral tumors. The rates of overall survival and recurrence-free survival at 5 years were 87.0% and 81.8%, respectively. There was no surgical mortality at 30 days. On univariate analysis, lymph node metastasis ($p = 0.0000$), nonlepidic predominant histologic subtype ($p = 0.0018$), and a solid appearance of the largest tumor on computed tomography ($p = 0.0088$) were significantly related to poor overall survival. On multivariate analysis, bilateral distribution of tumors ($p = 0.031$), lymph node metastasis ($p = 0.004$), and sublobar resection ($p = 0.042$) were independent predictors of poor survival.

Conclusions. Surgery has good outcomes and should be aggressively performed for patients with SPLA. The evaluation of lymph node status has an important role in deciding whether surgery is indicated. Bilateral tumors are a predictor of poor outcomes, requiring that caution be exercised. Lobectomy has a high cure rate and should be performed whenever possible. However, sublobar resection should be considered for patients likely to have poor residual lung function postoperatively.

(Ann Thorac Surg 2014;98:1983–8)

Owing to recent progress in diagnostic imaging techniques and the increased use of computed tomography (CT), patients with a confirmed or suspected diagnosis of multiple lung cancers are occasionally encountered. Recent studies have reported that 2.6% to 7.9% of patients who undergo resection of non-small cell lung cancer (NSCLC) have synchronous lung cancers [1–6], and this trend is increasing [6]. Surgical resection has become the mainstay of treatment for synchronous lung cancers, but the 3-year survival rate broadly ranges from 40% to 92% [1–3, 7–10]. The wide variability in outcomes is attributed not only to differences in treatment timing and demographic characteristics of patients, but also to the lack of standard criteria for differential diagnosis from intrapulmonary metastasis and for the selection of surgical procedures.

The seventh edition of the American Joint Committee on Cancer tumor-node-metastasis (TNM) staging system defined the presence of additional tumor nodules in the same lobe as T3 M0 and the presence of additional tumor nodules in other ipsilateral lobes as T4 M0, whereas nodules in the contralateral lung were defined as M1a disease [11]. Such nodules are generally regarded to be intrapulmonary metastases from the primary tumor, but may include separately staged synchronous primary lung cancers that require surgical resection. If multiple lung cancers are of different histologic types, differential diagnosis is relatively straightforward. However, if the histologic type is the same, the differential diagnosis of synchronous primary lung cancers and intrapulmonary metastasis remains challenging.

Recent studies have reported that multiple adenocarcinomas account for 40.3% to 91.3% of synchronous primary lung cancers [3–5, 8–10], making the differential diagnosis of multiple adenocarcinomas particularly important. To date, however, few studies have specifically focused on the diagnosis and surgical outcomes of synchronous primary lung adenocarcinomas (SPLA). Given progress in diagnostic imaging techniques and adenocarcinoma classification systems, we analyzed surgical outcomes in a recent series of patients with SPLA. Our main objective was to define appropriate methods and criteria for diagnosis and selection of surgical procedures on the basis of the outcomes of surgical therapy for patients with SPLA.

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Patients and Methods

This retrospective study was approved by the Ethics Committee of Kanagawa Cancer Center. Among 2,041 consecutive patients with primary lung cancer who underwent surgical resection in our hospital from April 1995 through December 2009, we studied patients with a pathologically confirmed diagnosis of SPLA who underwent complete surgical resection. Patients who had adenocarcinoma admixed with other histologic types were excluded from the study.

Preoperative evaluation included chest radiography, thin-section CT (TSCT) of the chest and upper abdomen, and positron emission tomography-CT. An expert consensus meeting attended by specialists from the fields of respiratory medicine, surgery, and diagnostic radiology was convened to evaluate preoperative findings. Patients with clinical (c) N2 disease were excluded from surgery. If patients had a mediastinal lymph node 1 cm or greater on the short axis on TSCT and a positive finding was identified on positron emission tomography-CT, mediastinoscopy or endobronchial ultrasonography was performed for histologic confirmation. Actually, because all patients in this study were cN0 or N1, no patients were offered endobronchial ultrasonography or mediastinoscopy.

Imaging features of the tumors, the presence or absence of ground-glass opacity (GGO), and tumor disappearance rates (TDR) were evaluated on TSCT. To calculate TDR, the maximum tumor diameter was measured on the lung window image (A) and the mediastinal window image (B), and TDR was calculated by the following formula: $(A - B) / A \times 100$ [12]. In our study, the CT features of tumors were classified into the following three subgroups according to the imaging features of the tumors and the TDR: pure GGO, entire nodules show GGO with a TDR of 100%; mixed GGO, nodules show some consolidation in GGO with a TDR of more than 25%; and solid, nodules consist mainly of consolidation with a TDR of 25% or less.

The same team of surgeons performed all resections. Surgical procedures were selected based on the size, location, and TSCT features of tumors, as well as performance status and pulmonary function. Solid and mixed GGO tumors were usually resected by lobectomy and pure GGO tumors by segmentectomy or wedge resection. For patients with poor pulmonary reserve or performance status, segmentectomy or wedge resection was selected instead of lobectomy.

The pathologic criteria for diagnosis of SPLA in our hospital are based on the Martini-Melamed criteria [13] and incorporate elements of the new international multidisciplinary lung adenocarcinoma classification [14] (Table 1). Patients with adenocarcinoma in situ and those with minimally invasive adenocarcinoma were included in analysis, but those with atypical adenomatous hyperplasia were excluded. For the analysis of survival rates according to the histologic type, adenocarcinoma in situ, minimally invasive adenocarcinoma, and the lepidic predominant subtype of invasive adenocarcinoma were included in the "lepidic predominant" subtype.

The disease stage was reclassified according to the seventh edition of the TNM classification [15]. Each tumor was staged, and the most advanced disease stage of all tumors was used as the disease stage of the patient. Tumor size on pathologic examination and CT features of the largest tumor and second tumor were included in the analysis. Postoperative adjuvant chemotherapy was mainly indicated for patients with pathologic (p) stage II or more advanced disease, and data on these patients were included in analysis.

Table 1. Pathologic Criteria for Diagnosis of Synchronous Primary Lung Adenocarcinomas

1. Major histologic subtypes of tumors are significantly different.
2. Major histologic subtypes are similar, but all tumors have lepidic growth component to a certain proportion, or immunohistologic features or genetic profiles of tumors are different.

Overall survival (OS) and recurrence-free survival were defined, respectively, as the time from initial surgery to the date of death and the date of recurrence or the final follow-up visit. Survival curves were calculated by the Kaplan-Meier method, and log rank tests were used for univariate analysis. Multivariate analysis was performed using a Cox proportional hazards model. Clinicopathologic features were compared according to tumor distribution with the use of Pearson's χ^2 test. All p values of less than 0.05 were considered to indicate statistical significance. All statistical analyses were performed using SPSS 11.0.1 software (SPSS, Chicago, IL).

Results

During the study period, synchronous primary lung cancers were diagnosed in 111 patients. Of these patients, 93 (4.6% of 2,041 patients undergoing resection of NSCLC) with SPLA were included in the study. The other 18 patients had other histologic types of tumors—adenocarcinoma plus squamous cell carcinoma in 8, squamous cell carcinoma plus squamous cell carcinoma in 3, adenocarcinoma plus other histologic types of cancer (large cell neuroendocrine carcinoma, pleomorphic carcinoma, and so forth) in 3, squamous cell carcinoma plus other types of cancer in 3, and a mixture of other histologic types in 1—and were excluded.

Patient Demographics

Demographic characteristics of the patients are shown in Table 2. Median age at the time of initial surgery was 68 years. There were 36 men (39%) and 33 smokers (36%). We confirmed that SPLA is more common among women and nonsmokers than lung cancer in general. The preoperative serum carcinoembryonic antigen level was elevated ($\geq$ 5.0 ng/mL) in 13 patients (14%).

Tumor Characteristics

Tumor characteristics are shown in Table 2. The number of tumors was 2 in 71 patients (76%), 3 in 18 patients (19%), and 4 or 5 in 4 patients (4%). The size of the largest tumor ranged from 10 mm to 57 mm (median 23). The tumor distribution was ipsilateral in 67 patients (same lobe, 31; different lobes of the ipsilateral lung, 36) and bilateral in 26.

Table 2. Patient Characteristics and Clinicopathologic Features

Characteristics	n (%) or Median (range)
Age	68 (49–84)
Sex	
Male	36 (39)
Female	57 (61)
Smoking status	
Current and former	33 (36)
Never	60 (65)
Preoperative CEA elevation, ≥ 5.0 ng/mL	13 (14)
Number of tumors	
2	71 (76)
3	18 (19)
4 or 5	4 (4)
Size of the largest tumor, mm	23 (10–57)
Distribution of tumors	
Ipsilateral same lobe	31 (33)
Ipsilateral different lobe	36 (39)
Bilateral	26 (28)
CT features of tumors, largest + second	
Solid + solid	11 (12)
Solid + mixed GGO	11 (12)
Solid + pure GGO	8 (9)
Mixed GGO + solid	8 (9)
Mixed GGO + mixed GGO	24 (26)
Mixed GGO + pure GGO	26 (28)
Pure GGO + mixed GGO	2 (2)
Pure GGO + pure GGO	3 (3)
Clinical stage	
I	87 (94)
II	5 (5)
IIIA[a]	1 (1)
Pathologic stage	
I	75 (81)
II	9 (10)
IIIA[b]	9 (10)
Histologic subtypes of the largest tumor	
Lepidic predominant[c]	63 (68)
Acinar predominant	10 (11)
Papillary predominant	10 (11)
Micropapillary predominant	3 (3)
Solid predominant	7 (8)

[a] One patient was cT3N1. [b] Eight patients were pT1-2N2 and 1 patient was pT3N1. [c] Adenocarcinoma in situ (n = 24) and minimally invasive adenocarcinoma (n = 11) were included.

CEA = carcinoembryonic antigen; CT = computed tomography; GGO = ground-glass opacity.

The CT features of the largest tumor were mixed GGO in 58 patients, solid pattern in 30, and pure GGO in 5. One or more solid lesions were present in 38 patients (41%).

Pathologic Findings

Pathologic findings are also shown in Table 2. The histologic subtype of the largest tumor was lepidic predominant in 63 patients (68%). The 1 patient with c-stage IIIA disease had T3 N1 cancer. Lymph node metastasis was found in 18 patients (19%); 10 had pN1 disease, and 8 had pN2 disease.

Surgical Procedures

Among the 26 patients with bilateral tumors, one-stage bilateral operations were performed in 6 patients, and two-stage bilateral operations in 20 (Table 3). One patient (1%) underwent pneumonectomy. Sublobar resection (wedge resection and segmentectomy) was included in treatment procedures for 54 patients (58%).

Surgical Outcomes

The follow-up period ranged from 8.1 to 198.1 months (median 56.0). At final follow-up, 12 patients (13%) had died, and 81 (87%) were alive. There was no perioperative death. Twelve patients died in the late phase; the cause of death was lung cancer in 9 patients, postoperative chronic empyema in 1, flare-up of tuberculosis in 1, and unknown in 1. The 3-year and 5-year OS rates were 93.6% and 87.0%, respectively (Fig 1). Recurrence developed in 17 patients (18%). The initial site of recurrence was intrapulmonary metastasis in 7 patients, lymph node metastasis in 5, distant metastasis in 3, pleural dissemination in 1, and recurrence at the resection stump in 1. All patients with pN2 disease had recurrence. The 3-year and 5-year recurrence-free survival rates were 89.2% and 81.8%, respectively (Fig 1).

Table 4 shows the results of univariate analysis of factors related to OS. The presence of lymph node metastasis ($p = 0.0000$), a nonlepidic predominant subtype of the largest tumor ($p = 0.0018$), and solid CT features of the largest tumor ($p = 0.0088$) were significantly related to poor outcomes. Bilateral tumors ($p = 0.0950$) and pathologic T2 to T3 disease ($p = 0.0885$) were slightly, but not significantly, related to poor outcomes. On multivariate analysis including the surgical procedure and tumor distribution in addition to the three significant factors identified on univariate analysis, the presence of lymph node metastasis

Table 3. Operative Details (n = 93)

Distribution and Type of Resection	n
Ipsilateral	
Pneumonectomy	1
Bilobectomy	5
Lobectomy	28
Lobectomy + segmentectomy	3
Lobectomy + wedge	14
Segmentectomy + segmentectomy	1
Segmentectomy + wedge	4
Multiple wedges	11
Bilateral	
Lobectomy + lobectomy	5
Lobectomy + segmentectomy	5
Lobectomy + wedge	5
Segmentectomy + wedge	5
Multiple wedges	6

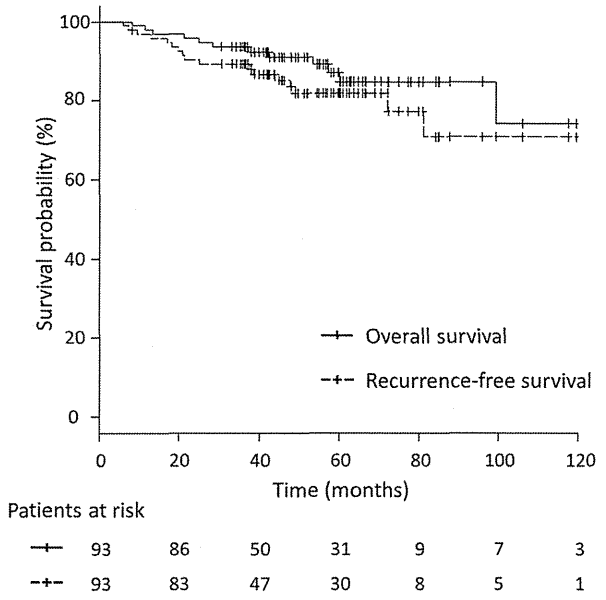

Fig 1. Overall survival (solid line) and recurrence-free survival (dashed line) of 93 patients with synchronous primary lung adenocarcinomas who underwent surgical resection. Five-year rates were 87.0% and 81.8% for overall and recurrence-free survival, respectively.

($p = 0.004$), bilateral distribution of tumors ($p = 0.031$), and the use of sublobar resection ($p = 0.042$) were independent predictors of poor survival (Table 5).

Comment

When we encounter patients with multiple tumors in the lung in clinical practice, it is extremely difficult to distinguish SPLA from intrapulmonary metastasis. The Martini-Melamed criteria [13] have been widely used for differential diagnosis in previous studies. Tumors of the same histologic type that arise in the same segment of the lung are diagnosed as intrapulmonary metastasis. Even if different segments are involved, tumors of the same histologic subtype are diagnosed as intrapulmonary metastasis if metastasis is found at shared lymphatic pathways. However, in the current era of genetic analysis of factors such as epidermal growth factor receptor, SPLA involving multiple lobes of the same lung and accompanied by mediastinal lymph node metastasis have been reported [16]. In addition to the Martini-Melamed criteria, it is therefore necessary to evaluate other factors for diagnosis of this type of cancer. In patients with multiple adenocarcinomas, the histologic subtypes of the tumors must be considered. The recently proposed comprehensive histologic assessment has facilitated the differential diagnosis of multiple primary NSCLC and metastases [17]. However, the problem remains that lepidic predominant primary tumors are likely to be diagnosed as intrapulmonary metastasis if the histologic subtype ratio is similar. Recently, there has been an increasing trend in multiple tumors showing GGO, particularly among nonsmoking women in Asia. Such lesions are likely to be

Table 4. Univariate Analysis of Predictors of Survival

Predictors	n	Overall 5-Year Survival	*p* Value
Age, years			
< 70	53	83.8%	0.9152
≥ 70	40	92.0%	
Sex			
Male	36	77.4%	0.1265
Female	57	93.1%	
Smoker			
Current and former	33	81.8%	0.6533
Never	60	90.0%	
Preoperative serum CEA, ng/mL			
< 5.0	80	90.0%	0.2167
≥ 5.0	13	68.4%	
Size of the largest tumor, mm			
≤ 30	72	84.5%	0.2365
> 30	21	95.2%	
CT features of the largest tumor			
Solid	30	75.6%	0.0088
Mixed and pure GGO	63	92.5%	
Distribution of tumors			
Ipsilateral	67	90.9%	0.0950
Bilateral	26	76.9%	
Number of tumors			
2	71	90.7%	0.3327
≥ 3	22	71.1%	
Highest pT			
T1	66	90.7%	0.0885
T2–3	27	78.6%	
Highest pN			
N0	75	93.4%	0.0000
N1	10	75.0%	
N2	8	41.7%	
Surgical procedures			
Lobectomy	39	92.5%	0.5086
Sublobar included			
Segmentectomy[a]	18	82.1%	
Wedge resection	36	80.4%	
Mediastinal LN management			
Systematic dissection	36	83.3%	0.9118
Sampling	20	80.0%	
Not dissected	37	88.5%	
Histologic subtype of the largest tumor			
Lepidic predominant	63	98.4%	0.0018
Nonlepidic predominant	30	66.9%	
Adjuvant chemotherapy[b]			
Yes	6	83.3%	0.7050
No	12	50.0%	

[a] Nine patients who underwent segmentectomy and wedge resection were included. [b] Eighteen patients with p-stage II or greater disease were included.

CEA = carcinoembryonic antigen; CT = computed tomography; GGO = ground-glass opacity; LN = lymph node.

Table 5. Multivariate Analysis of Predictors of Survival

Variables	n	*p* Value	Hazard Ratio (95% CI)
CT features of the largest tumor			
Solid/mixed and pure GGO	30/63	0.200	0.421 (0.112–1.582)
Distribution of tumors			
Bilateral/ipsilateral	26/67	0.031	4.630 (1.148–18.666)
Lymph node involvement			
Yes/no	18/75	0.004	10.560 (2.142–52.076)
Use of sublobar resection			
Yes/no	54/39	0.042	4.425 (1.054–18.580)
Predominant histology			
Lepidic/nonlepidic	63/30	0.261	2.395 (0.552–10.982)

CI = confidence interval; CT = computed tomography; GGO = ground-glass opacity.

classified as intrapulmonary metastasis. However, tumors with a high GGO ratio are most likely not intrapulmonary metastasis [18]. The diagnostic criteria for multiple lung adenocarcinomas in our hospital have taken this point into account. The good treatment outcomes in our study might have been attributed to the exclusion of patients with intrapulmonary metastasis, which is associated with a poor prognosis.

To our knowledge, this is the second largest, relatively long-term follow-up study of surgical outcomes in patients with SPLA [8]. The 5-year OS rate in our study was 87.0%, which is considered extremely good. Several factors were related to outcomes, and lymph node metastasis had the greatest impact. Previous studies have similarly reported that the presence or absence of lymph node metastasis is a significant prognostic factor [2, 3, 8, 10, 19, 20]. In our study, however, 5-year survival rates were 75.0% for patients with pN1 disease and 41.7% for patients with pN2 disease, better rates than those reported by the International Association for the Study of Lung Cancer lung cancer staging project (38% for pN1 disease, 22% for pN2 disease) [21]. The specialized design of our study in patients with adenocarcinoma might have contributed to better outcomes.

The Martini-Melamed criteria classify cases with N2 nodal involvement as intrapulmonary metastasis, but not multiple cancers. Some studies have excluded patients with N2 disease from the analysis of surgical outcomes [5, 9, 22]. In contrast, because we performed detailed histologic subtyping synchronous primary lung cancers could be diagnosed even in the presence of N2 disease. We, therefore, could obtain a better overall picture of the outcomes of surgical treatment for synchronous primary lung cancers.

Curative chemoradiotherapy is basically indicated for patients with cN2 disease. In our study, 8 cases of pN2 disease (ipsilateral, 4; bilateral, 4) were detected by chance on postoperative pathologic examination. Unexpected pN2 disease was thus detected in approximately 10% of patients, a finding that is generally consistent with the findings of previous studies. Of these patients with bilateral disease, pN2 disease was diagnosed on the second of two-stage bilateral resections in 2 patients and on one-stage bilateral surgery in 1 patient. For patients who underwent two-stage surgery, the side with more advanced lesions or with lesions likely to negatively affect outcomes is usually initially resected. In fact, however, half of all more advanced lesions were not resected at the first operation.

Synchronous surgery for bilateral tumors is considered an effective strategy for preventing disease progression and delays in adjuvant therapy in patients with clinical N0 to pathologic N2 disease. However, synchronous bilateral lobectomy with lymph node dissection is associated with increased risk and therefore should only be performed in carefully selected patients. Given the treatment outcomes in patients with pN2 disease, if N2 disease is detected at the first stage of two-stage resection, treatment options such as chemotherapy and stereotactic body radiotherapy should be also considered instead of performing lobectomy at the second stage.

The relations between surgical procedures and outcomes have been extensively studied. A number of studies have reported no difference in survival according to whether sublobar resection was performed [3, 5, 6, 10, 22]. In our study, sublobar resection was a significant independent predictor of poor outcomes on multivariate analysis. This result is attributed to a negative impact of sublobar resection on curability. In our study, 59% of the patients had tumors with a high GGO ratio, which are associated with relatively good outcomes. The latest American College of Chest Physicians evidence-based clinical practice guidelines recommend that these lesions should be handled separately as multifocal lung cancer and patients should undergo sublobar resection because single tumors with a high GGO ratio have good outcomes [18]. However, clear-cut criteria defining lesions that should be treated by sublobar resection are currently unavailable. Imaging findings of tumors may be useful for determining the range of resection. As shown in our study and previous reports [12, 23, 24], mixed or pure GGO lesions had a high TDR and good outcomes, whereas solid lesions were associated with poor outcomes. Therefore, solid lesions should be treated by radical lobectomy if permitted by lung function.

Interestingly, bilateral tumors were an independent predictor of poor outcomes in our study. Previous studies have reported that OS does not differ significantly according to tumor distribution [3, 8–10]. In contrast to our results, some studies reported that bilateral tumors were associated with significantly better outcomes [2, 20]. To investigate reasons for the poorer outcomes in patients with bilateral tumors, we studied differences in clinicopathologic factors related to tumor distribution. Bilateral tumors were found to be associated with a higher preoperative carcinoembryonic antigen level, a greater number of tumors, a larger size of the second tumor, and a higher proportion of patients who underwent sublobar resection (Table 6). These findings indicate that many of our subjects with bilateral tumors had aggressive disease, and this factor might have led to the difference in outcomes. Moreover, because patients with bilateral tumors had many lesions, it was difficult to perform lobectomy for all lesions. This factor may have also contributed to poorer outcomes.

Table 6. Correlation Between Tumor Distribution and Other Clinicopathologic Features

Variables	Ipsilateral	Bilateral	*p* Value
CT features of the largest tumor			
Solid	22	8	1.000
Mixed and pure GGO	45	18	
Preoperative serum CEA, ng/mL			
< 5.0	61	19	0.032
≥ 5.0	6	7	
Tumor size of the largest tumor, mm			
≤ 30	47	21	0.221
> 30	20	5	
Tumor size of the second tumor, mm			
≤ 20	62	18	0.007
> 20	5	8	
Number of tumors			
2	56	15	0.011
≥ 3	11	11	
Pathologic stage			
Stage I	53	22	0.388
Stage II–III	14	4	
Highest pN			
N0–1	63	22	0.213
N2	4	4	
Surgical procedures			
Lobectomy	34	5	0.009
Sublobar included	33	21	

CEA = carcinoembryonic antigen; CT = computed tomography; GGO = ground-glass opacity.

Finally, our study had several limitations. First, patient selection was biased because this was a single-center, retrospective study. Second, molecular phenotype such as epidermal growth factor receptor mutation was not assessed in all patients at the diagnosis of multiple lung adenocarcinomas. Finally, we did not compare patients with SPLA who underwent surgery with patients who did not undergo surgery or with patients who underwent only incomplete resection. However, because our study was a single-center trial, treatment policy, surgical procedures, postoperative care, and histopathologic evaluations were standardized. We believe that these conditions led to high-quality data.

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Original Research

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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.

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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.