Table 3 Multivariate cox proportional hazards model analyses of various factors affecting overall survival in primary, resected stage IA NSCLC

Variable	Total			Male			Female		
	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*
Sex, male vs. female	1.422	1.179–1.715	0.0002	–	–	–	–	–	–
Age, ≥70 years vs. <70 years	1.548	1.325–1.808	<0.0001	1.605	1.333–1.605	<0.0001	1.470	1.106–1.470	0.0080
Performance status, ≥1 vs. 0	1.727	1.461–2.037	<0.0001	1.869	1.545–1.869	<0.0001	1.383	0.994–1.926	0.0543
Pack-years, ≥40 vs. <40	1.129	0.948–1.344	0.1742	1.148	0.955–1.379	0.1410	0.841	0.463–1.526	0.5697
Histologic type[a], squamous vs. nonsquamous	1.080	0.909–1.284	0.3807	1.168	0.973–1.401	0.0950	1.697	1.049–2.739	0.0309

[a] Nonsquamous cell carcinoma is comprised of adenocarcinoma and large cell carcinoma.

Table 4 Multivariate cox proportional hazards model analyses of various factors affecting disease-specific survival in primary, resected stage IA NSCLC

Variable	Total			Male			Female		
	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*
Sex, male vs. female	1.325	1.054–1.664	0.0161	–	–	–	–	–	–
Age, ≥70 years vs. <70 years	1.314	1.080–1.597	0.0063	1.350	1.068–1.706	0.0120	1.265	0.886–1.808	0.1949
Performance status, ≥1 vs. 0	1.387	1.119–1.718	0.0029	1.475	1.151–1.890	0.0022	1.088	0.705–1.680	0.7018
Pack-years, ≥40 vs. <40	1.218	0.981–1.511	0.0741	1.263	1.004–1.587	0.0463	0.768	0.351–1.675	0.5069
Histologic type[a], squamous vs. nonsquamous	1.098	0.886–1.360	0.3935	1.160	0.926–1.455	0.1971	1.517	0.810–2.849	0.1926

[a] Nonsquamous cell carcinoma is comprised of adenocarcinoma and large cell carcinoma.

ation between smoking history and lung cancer survival [17,18]; however, these studies did not utilize multivariate analysis. Harpole et al. reported a multivariate model that quantified the risk of recurrence and cancer death for patients with stage I NSCLC, but they demonstrated no significant impact on univariate analysis using smoking history [7]. Some other studies showed smoking history is negative prognostic factor [19,20]. However, their studies included relatively small cases and therefore may be insufficient power to detect smoking effects.

Hinds et al. reported model-predicted survival curves in women for smokers and never-smokers after adjustments for age, disease stage at diagnosis, and tumor histology [8]. The curves were significantly different. But, they did not use the Cox proportional hazards model and did not have information on pretreatment PS. Sobue et al. reported that current smokers who smoked 50 pack-years or more showed a 2.38 times higher risk of death than non-smokers for patients who undergo operations for adenocarcinoma of the lung [10]. Isobe et al. reported similar results [11]. In contrast, Sioris et al. showed that smoking history is one of the prognostic factors in squamous cell carcinoma for overall survival but not in adenocarcinoma [13]. Fujisawa et al. provided answer to this conflict. They employed multivariate analysis and showed smoking history is prognostic factor in evaluating overall long-term survival in patients with stage I primary resected NSCLC [14]. Nevertheless, they did not show important information on pretreatment PS. Furthermore, smoking history is not prognostic factor in evaluating disease-specific survival. It has been said that comorbidity may be one of the most important prognostic factor [22], as smoking is strongly associated with numerous serious disease such as chronic obstructive pulmonary disease, coronary heart disease, and stroke. To offset the influence of comorbidities on survival, disease-specific survival is superior to overall survival. On the other hand, the large number of patients at the multicenter gave us considerable confidence in the reliability of our data on smoking history and prognosis.

In our study, smoking status was investigated only at the time of admission to the hospital, and no information on smoking status was obtained after the operation. Richardson et al. reported that patients treated for SCLC who continue to smoke cigarettes increase their rate of developing second lung cancers [23]. In Japan, Kawahara et al. reported the same results [24]. In NSCLC, Fujisawa et al. reported no significant differences between postoperative smoking status and outcome in the population of patients, alive or dead, due to recurrent disease, second malignancy, or non-malignant disease [14]. Further study is necessary to determine the prognosis and incidence of recurrence among patients who continue to smoke.

Although the mechanisms by which smoking affects the prognosis of lung cancer patients independently of other factors are not yet clear, some recent reports on oncogene suggest the clinical influence of cigarette smoking. Molecular changes that have been demonstrated in lung cancer include the activation of oncogenes such as ras, myc, bcl-2, and c-erbB-2, and the loss of tumor suppressor genes such as p53, RB and $p16^{INK4\alpha}$ [25–27]. Recently, Vahakangas et al. reported that p53 mutations occur more commonly in smokers and ex-smokers than in never-smokers [28]. Furthermore, Tammemagi et al. reported that p53 alteration and smoking history are negative prognostic factor [15]. Heavy smokers may have these molecular changes. These reports support our data showing poor prognosis in heavy smokers.

Among patients with resectable tumors, advanced age is generally described as an unfavorable prognostic factor, possibly because of higher postoperative mortality rates [29]. Our findings confirmed that age is a prognostic factor in a curative resection setting.

Gender and smoking history were closely correlated in Japan. In this series, we observed striking differences in smoking history between men and women; that is, 82.5% of women were nonsmokers and 88.2% of men were smokers. Furthermore, smokers with more than 40 pack-years made up only 5.1% of all females and 58.6% of all males. That is, mean cigarette consumption was significantly lower in smoking women than in men. Such differences in smoking history likely resulted in different clinical presentation, histology, and treatment. It has been reported that the differences in smoking history may explain the better prognosis in females [8,30]. That is to say, gender is potential confounding factor in this study. In order to avoid systemic bias, we analyzed subgroup analyses.

In conclusion, the results of the current study found a preoperative smoking history to be a significant predictor of prognosis by univariate and multivariate analyses, in males. We showed a significant correlation between cigarette smoking and long-term disease-specific survival in stage IA NSCLC patients in numerous cases. The poor prognosis makes patients with smoking history an important population for creating stratification levels in clin-

ical trials and for the study of chemoprevention or smoking cessation study.

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EGFR Mutation Status in Japanese Lung Cancer Patients: Genotyping Analysis Using LightCycler

Hidefumi Sasaki,[1,2] Katsuhiko Endo,[1] Akimitsu Konishi,[1] Minoru Takada,[3] Masaaki Kawahara,[3] Keiji Iuchi,[2] Akihide Matsumura,[2] Meinoshin Okumura,[2] Hisaichi Tanaka,[2] Tomoya Kawaguchi,[3] Toshiki Shimizu,[3] Hiroshi Takeuchi,[3] Motoki Yano,[1] Ichiro Fukai,[1] and Yoshitaka Fujii[1]

Abstract

Purpose: Recently, somatic mutations of the epidermal growth factor receptor (*EGFR*) gene were found in ~25% of Japanese lung cancer patients. These *EGFR* mutations are reported to be correlated with clinical response to gefitinib therapy. However, DNA sequencing using the PCR methods described to date is time-consuming and requires significant quantities of DNA; thus, this existing approach is not suitable for a routine pretherapeutic screening program.

Experimental Design: We have genotyped *EGFR* mutation status in Japanese lung cancer patients, including 102 surgically treated lung cancer cases from Nagoya City University Hospital and 16 gefitinib-treated lung cancer cases from Kinki-chuo Chest Medical Center. The presence or absence of three common *EGFR* mutations were analyzed by real-time quantitative PCR with mutation-specific sensor and anchor probes.

Results: In exon 21, *EGFR* mutations (CTG → CGG; L858R) were found from 8 of 102 patients from Nagoya and 1 of 16 from Kinki. We also detected the deletion mutations in exon 19 from 7 of 102 patients from Nagoya (all were deletion type 1a) and 4 of 16 patients from Kinki (one was type 1a and three were type 1b). In exon 18, one example of G719S mutation was found from both Nagoya and Kinki. The L858R mutation was significantly correlated with gender (women versus men, $P < 0.0001$), Brinkman index (600 ≦ versus 600>, $P = 0.001$), pathologic subtypes (adenocarcinoma versus nonadenocarcinoma, $P = 0.007$), and differentiation status of the lung cancers (well versus moderately or poorly, $P = 0.0439$), whereas the deletion mutants were not. *EGFR* gene status, including the type of *EGFR* somatic mutation, was correlated with sensitivity to gefitinib therapy. For example, some of our gefitinib-responsive patients had L858R or deletion type 1a mutations. On the other hand, one of our gefitinib-resistant patients had a G719S mutation.

Conclusions: Using the LightCycler PCR assay, the *EGFR* L858R mutation status might correlate with gender, pathologic subtypes, and gefitinib sensitivity of lung cancers. However, further genotyping studies are needed to confirm the mechanisms of *EGFR* mutations for the sensitivity or resistance of gefitinib therapy for the lung cancer.

Lung cancer is a major cause of death from malignant diseases because of its high incidence, malignant behavior, and lack of major advancements in treatment strategy (1). Lung cancer was the leading indication for respiratory surgery (42.2%) in 1998 in Japan (2). More than 15,000 patients underwent surgical operation at Japanese institutions in 1998 (2). The clinical behavior of the lung cancer is largely associated with its stage. The cure of the disease by surgery is only achieved in cases representing an early stage of lung cancer (3).

The epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitor, gefitinib, has been approved in Japan for the treatment of non-small cell lung cancer from 2002. Although *EGFR* is more abundantly expressed in lung carcinoma (4, 5), *EGFR* expression, as detected by immunohistochemistry, did not reveal any obvious relationship with response to gefitinib (6). Clinical trial have revealed significant variability in the response to gefitinib, with higher response in Japanese patients than in predominantly European-derived population (27.5% versus 10.4%; ref. 7). The partial clinical responses to gefitinib have been observed most frequently in women, in nonsmokers, and in patients with adenocarcinoma (8–10). More recently, we have collaborated with Dana-Farber Cancer Institute and found that novel *EGFR* mutations status at ATP binding pockets in Japanese non-small cell lung cancer patients were correlated with the clinicopathologic features related to good response to gefitinib (11). Actually, *EGFR* mutations in lung cancer have been correlated with clinical response to gefitinib therapy *in vivo* and *in vitro* (11–13).

Authors' Affiliations: [1]Department of Surgery II, Nagoya City University Medical School, Nagoya, Japan and Departments of [2]Surgery and [3]Internal Medicine, National Hospital Organization, Kinki-chuo Chest Medical Center, Sakai, Japan
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Requests for reprints: Hidefumi Sasaki, Department of Surgery II, Nagoya City University Medical School, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya, Aichi 467-8601, Japan. Phone: 81-52-853-8231; Fax: 81-52-853-6440; E-mail: hisasaki@med.nagoya-cu.ac.jp.

The standard for experimental detection of mutations is direct sequencing of DNA samples from the tissues. For known mutations, real-time polymerase chain detection followed by melting curve analysis, using hybridization probes, is highly sensitive, rapid, and an efficient alternative approach to mutation detection (14–16).

To determine the *EGFR* mutation status in Japanese lung carcinoma for screening and diagnostic purposes, we wanted to develop a faster and easy method to detect *EGFR* mutations. In this report, we investigated *EGFR* mutation status by the real-time reverse transcription-PCR assay using LightCycler (17) mutation-specific sensor and anchor probes. With this method, 32 samples were genotyped within 1 hour without the need of any post-PCR sample manipulation. The findings were compared with the clinicopathologic features of lung cancer.

Materials and Methods

Patients. The study group included 102 lung cancer patients who had undergone surgery (but did not receive gefitinib) at the Department of Surgery II, Nagoya City University Medical School, between 1997 and 2000. The study group also included 16 lung cancer patients who had undergone surgery at the Department of Surgery, National Hospital Organization, Kinki-chuo Chest Medical Center, and were subsequently treated with gefitinib. These 16 samples were sequenced by ABI prism 3100 analyzer (Applied Biosystems Japan, Ltd., Tokyo, Japan; data not shown) and analyzed by ABI prism Seq Scape version 2.1.1. The lung tumors were classified according to the general rule for clinical and pathologic record of lung cancer in Japan (18). All tumor samples were immediately frozen and stored at −80°C until assayed.

The clinical and pathologic characteristics of the 102 lung cancer patients are as follows: 52 cases at stage I, 16 at stage II, and 34 at stage III to IV. The mean age was 65.5 years (range, 42-85). Among the 102 lung cancer patients, 49 (48%) were diagnosed as having adenocarcinoma, 32 (31.4%) squamous cell carcinoma, 9 (8.8%) adenosquamous cell carcinoma, and 7 (6.9%) small cell carcinoma.

PCR assays for EGFR. The genomic DNA was extracted from lung cancer tissues and matched normal lymphocytes from the peripheral blood using the Wizard SV Genomic DNA purification system (Promega Corporation, Madison, WI). Initially, 58 DNA samples were also extracted from lung cancer tissues from Nagoya City University and sequenced as reported in our previous paper (11). These sets of DNA were used as a positive and negative control for genotyping. DNA concentration was determined by spectrophotometry and adjusted to a concentration of 50 ng/mL. We then used 1 μL of each DNA for LightCycler analyses. To ensure the fidelity of DNA extraction, all samples were subjected to PCR amplification with oligonucleotide primers specific for exon 18 of the *EGFR* gene and then digested by *Sac*I enzyme. The primer sequences for *EGFR* gene in exon 18 were as follows: the forward primer, 5-TCCAAATGAGCTGGCAAGTG-3, and the reverse primer, 5-TCCCAAACACTCAGTGAAACAAA-3 (397 bp). The cycling conditions were as follows: initial denaturation at 95°C for 15 minutes followed by 35 cycles at 95°C for 20 seconds, 57°C for 20 seconds, 72°C for 30 seconds, and one cycle of 72°C for 3 minutes. The products were purified by Qiagen PCR purification kit (Qiagen, Valencia, CA) and then digested with restriction enzyme at 37°C for 2 hours. The genotyping PCR reactions were done using LightCycler DNA Master Hybridization probes kit (Roche Molecular Biochemicals, Mannheim, Germany) in a 20 μL reaction volume. The primer sequences for *EGFR* gene in exon 18 were as follows: the forward primer, 5-TCCAATGAGCTGGCAAGTG-3, and the reverse primer, 5-TCCCAAACACTCAGTGAAACAAA-3 (397 bp). For the exon 18 genotyping, sensor (LC Red 640-GCACCGGAGCCCAGCA) and anchor (GCCAGGGACCTTATACACGTGCCGAA-Fluorescein) probes were used. The cycling conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 45 cycles at 95°C for 10 seconds, 60°C for 10 seconds, and 72°C for 16 seconds The primer sequences for *EGFR* gene in exon 19 were as follows: the forward primer, 5-CGTCTTCCTTCTCTCTCTGTC-3, and the reverse primer, 5-GACATGAGAAAAGGTGGGC-3 (175 bp). For the exon 19 genotyping, sensor (GCTATCAAAACATCTCC-Fluorescein) and anchor (LC Red 640-AAAGCCAACAAGGAAATCCTCGATGTGAGTTTCTGCTTTGCTGTGTGGGG) probes were used. The cycling conditions were as follows: initial denaturation at 95°C for 10 minutes followed by 45 cycles at 95°C for 10 seconds, 60°C for 10 seconds, and 72°C for 7 seconds. The primer sequences for *EGFR* gene in exon 21 were as follows: the forward primer, 5-GCTCAGAGCCTGGCATGAA-3, and the reverse primer, 5-CATCCTCCCCTGCATGTGT-3 (349 bp). The cycling conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 45 cycles at 95°C for 10 seconds, 57°C for 10 seconds, and 72°C for 14 seconds. For the exon 21 genotyping, sensor (Fluorescein-AGTTTGGCCCGCCCA) and anchor (LC Red 640-CCTCCTTCTGCATGGTATTCTTTCTCTTCCGCACCCAG) probes were used.

Statistical methods. Statistical analyses were done using the Mann-Whitney *U* test for unpaired samples and Wilcoxon's singed rank test for paired samples. Linear relationships between variables were determined by simple linear regression. Correlation coefficients were determined by rank correlation using Spearman's test and χ^2 test. The overall survival of lung cancer patients was examined by the Kaplan-Meier methods and differences were examined by the log-rank test, Breslow-Gehan-Wilcoxon test, and Cox proportional hazard regression model. All analyses were done using the StatView software package (Abacus Concepts, Inc., Berkeley, CA) and results were considered significant when $P < 0.05$.

Results

Fidelity of allele-specific PCR confirmed by conventional PCR assay in lung cancer tissues. Using the exon 18 primer sets, a PCR product of 397 bp was obtained. We have analyzed the product using PCR-RFLP method. The wild-type DNA does not have a *Sac*I site within the 397 bp. The PCR products digested with *Sac*I were loaded with 2% agarose gel and wild-type DNA should be visualized as one band. However, if the substitution mutation G719S were present, the PCR products digested by *Sac*I will be visualized as three bands. Using this method, PCR products were visualized from all lung cancer patients studied. In exon 18, a G719S mutation was found from one Nagoya specimen (stage Ia, well-differentiated adenocarcinoma with bronchioloalveolar carcinoma pattern at the edge of tumor, female, nonsmoker patient) and one Kinki specimen (Fig. 1A). These mutants were also analyzed by LightCycler. The anchor probe was matched for wild type. As shown in Fig. 1B for the G719S mutation in exon 18, the homozygous wild-type PCR product showed a single peak at 69°C, whereas the heterozygous products (mutant) showed an additional peak at 59°C. The LightCycler method using the mutation-specific probes confirmed the results with the restriction fragment analysis.

Genotyping of EGFR at exon 19 and exon 21 in lung cancer tissues. For exon 21 genotyping, the anchor probe was matched for L858R mutation. As shown in Fig. 2, for the L858R mutation in exon 21, the homozygous wild-type PCR product showed a single peak at 53°C, whereas the heterozygous products (mutant) showed an additional peak at 65°C. From the 102 lung cancer patients, 8 patients had the L858R mutation. One was male and seven were female. Seven were nonsmokers and one was a smoker (Brinkman index was 600). All eight patients had adenocarcinoma, one was moderately differentiated, and

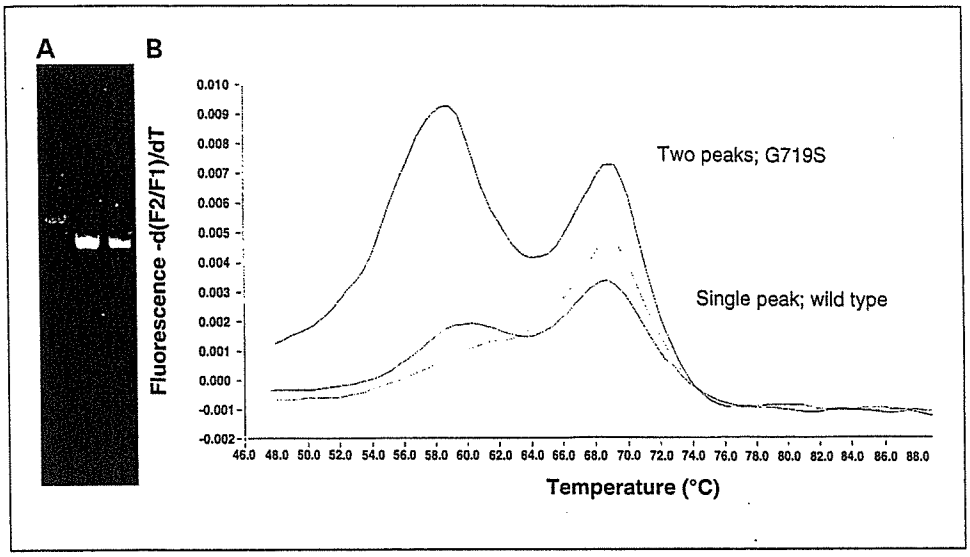

Fig. 1. *A*, analyzed data using PCR-RFLP. Left lane, the wild-type DNA within the 397 bp does not have *Sac*I site. The PCR products restricted with *Sac*I were loaded with 2% agarose gel and was visualized as one band. Right lane, the substitution mutation G719S caused *Sac*I site, and the PCR products restricted by *Sac*I was visualized as three bands. *B*, detection of a G719S mutation in the *EGFR* gene in genomic DNA extracted from lung cancer tissues. The negative derivative of the fluorescence ($-dF / dT$) versus temperature graph shows peaks with different T_m. The wild-type sample showed a single T_m at 69°C. The heterozygous mutant sample showed an additional peak at 59°C.

seven were well differentiated. Five of eight adenocarcinomas showed bronchioloalveolar carcinoma pattern at the edge of tumor. Thus, L858R mutation status was significantly correlated with gender, Brinkman index, pathologic subtypes, and differentiation of lung cancer (Table 1). Eight of eight PCR products from matched peripheral lymphocyte DNA showed a single peak, suggesting that the mutations were somatic. L858R mutation was also found in one nonsmoking female adenocarcinoma patient from Kinki-chuo Chest Medical Center.

For exon 19 genotyping, the anchor probe was matched for deletion type 1a (2,235-2,249 nucleotides deletion; deletion GGAATTAAGAGAAGC) mutation. As shown in Fig. 3, for the deletion 1a mutation in exon 19, the PCR product showed a single peak at 56°C, whereas the deletion 1b products (2,236-2,250 nucleotides deletion; deletion GAATTAAGAGAAGCA) showed a peak at 47°C. From the 102 lung cancer patients, seven patients had the deletion 1a mutation. Four were males and three were females. Three were nonsmokers and four were smokers. Four patients had adenocarcinoma, two had squamous cell carcinoma, and one had adenosquamous cell carcinoma. One of the tumors was moderately differentiated, two were poorly differentiated, and three were well differentiated. One of four adenocarcinomas showed bronchioloalveolar carcinoma pattern at the edge of tumor. Thus, deletion 1a mutation status was not significantly correlated with gender, Brinkman index, pathologic subtypes, and differentiation of lung cancer (Table 2). Five of seven PCR products from matched peripheral lymphocyte DNA were available and showed a single peak, suggesting that these mutations were somatic.

The mutations detected in lung cancer specimens from Kinki-chuo Chest Medical Center are summarized in Table 3. L858R mutation and deletion type 1a were found from partial response patients. On the other hand, G719S mutation was found from a patient with no response to gefitinib (progressive disease). A total of six mutations were found from 16 gefitinib-treated patients (37.5%). Taken together, 22 mutations were found from 117 examined samples in our analysis (18.8%).

The overall survival of 102 lung cancer patients from Nagoya City University, with follow-up through December 30, 2003, was studied in reference to the *EGFR* mutation status. There was no significant difference in the prognosis between the patients with wild-type *EGFR* (n = 86, 22 were dead) and the patients with

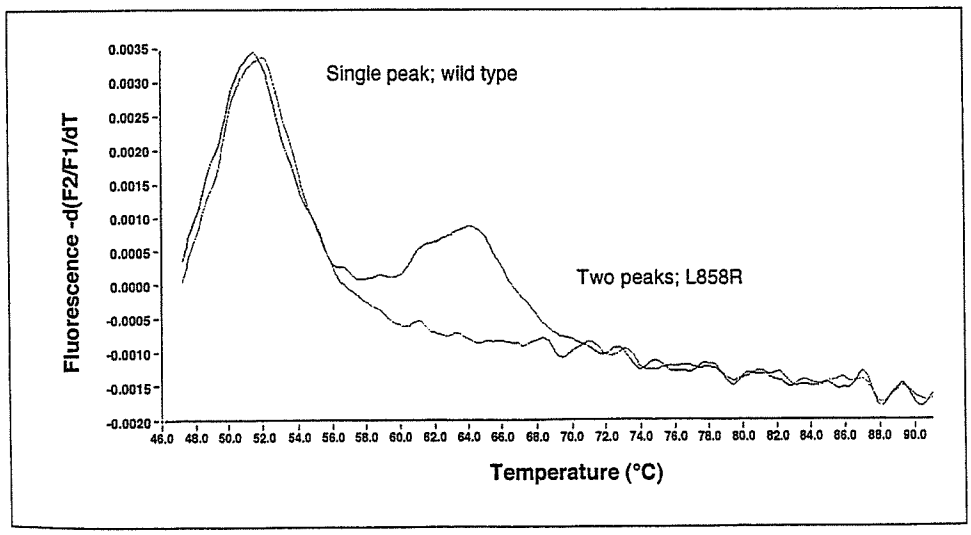

Fig. 2. The L858R mutation in exon 21 of the homozygous wild-type PCR product showed a single peak at 53°C, whereas the heterozygous products (mutant) showed an additional peak at 65°C.

Table 1. Clinicopathologic data of 102 lung cancer patients

Factors	L858R Mutation patients (%)	L858R Wild-type patients (%)	*P*
Mean age (y), 65.5 ± 9.3	8	94	
Stage			
I	7 (87.5)	45 (47.9)	0.0744
II-IV	1 (12.5)	49 (52.1)	
Lymph node metastasis			
N0	7 (87.5)	60 (63.8)	0.3341
N+	1 (12.5)	34 (36.2)	
BI			
≦600	8 (100)	32 (34.0)	0.001
>600	0 (0)	62 (66.0)	
Differentiation			
Well	7 (87.5)	31 (43.1)	0.0439
Moderately or poorly	1 (12.5)	41 (56.9)	
Pathologic subtypes			
Adenocarcinoma	8 (100)	41 (43.6)	0.007
Nonadenocarcinoma	0 (0)	53 (56.4)	
Age			
≦60	2 (25.0)	26 (27.7)	0.9999
>60	6 (75.0)	68 (72.3)	
Gender			
Male	1 (12.5)	80 (85.1)	<0.0001
Female	7 (87.5)	14 (14.9)	

Abbreviations: N+, lymph node metastasis positive; BI, Brinkman index.

mutation in the *EGFR* gene (n = 16, two were dead; log-rank test, P = 0.3608; Breslow-Gehan-Wilcoxon test, P = 0.4761), although the observation period was short.

Discussion

We obtained findings that L858R *EGFR* mutation status was significantly correlated with gender, smoking history, and pathologic subtypes of lung cancers. This was in agreement with the recent reports that *EGFR* gene mutations are common in lung cancers from never smokers (13) and females with adenocarcinoma (11). Our analysis also suggested that the type of *EGFR* mutation might be correlated with the sensitivity of gefitinib therapy for lung cancers.

When the PCR is used for the detection of mutations in very small amounts of DNA, although we would like to start from biopsy samples in the future, it is usually necessary to use "nested PCR." In this case, a DNA fragment is amplified with a first set of primers and part of the product is reamplified with a second set of primers complementary to sequences in the product. Recent developments in fast PCR and real-time detection of products make a more sensitive approach to detection of mutations possible (14–16, 19). We have optimized mutation detection, without nested PCR, using the LightCycler. This instrument measures fluorescence during PCR and can detect the SYBR Green dye when it is intercalated in double-stranded DNA, allowing the detection of double-stranded PCR product formation. The use of labeled probes homologous to the PCR product permits specific identification of PCR products (17). In the LightCycler, two adjacent probes were used, labeled with different fluorescent molecules. When the probes were bound to the single-stranded target, one to five bases apart, the 3′-end label of the 5′ probes came close to the 5′-end label of the 3′ probe, resulting in resonance and strong fluorescence at a specific wavelength. An advantage of this strategy is that hybridization of the probe is not restricted to the temperature range required for Taq polymerase to remove a base (19, 20). Further melting curves can be produced after PCR to assess the dissociation temperature of the probe. Mutations covered by the probe can be detected by a shift in melting temperature. The one-cycle analysis took ~1 hour and could examine 32 samples.

Because so many *EGFR* mutation phenotypes were discovered, it would be of interest to determine whether resistance to *EGFR* inhibition emerges through secondary mutation as is the case in imatinib-treated chronic myelogenous leukemia (21). Our data showed that L858R mutation and deletion type 1a were found in gefitinib-sensitive patients; on the other hand, a G719S mutation was found in a gefitinib-resistant patient. Interestingly, recent data reported that L858R mutant (transfected cell) was inhibited at 10-fold lower concentrations of tyrosine kinase inhibitor; however, the deletion mutant seemed to have similar sensitivities as wild-type *EGFR*

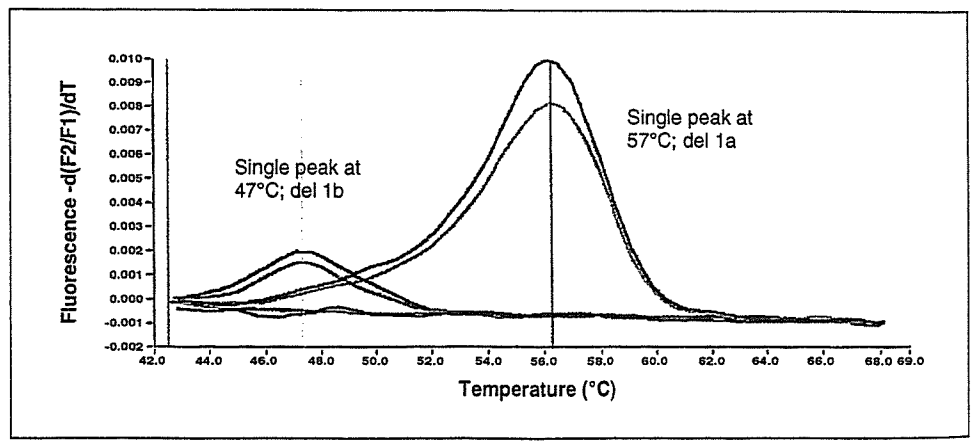

Fig. 3. Detection of the deletion mutations in the *EGFR* gene in genomic DNA extracted from lung cancer. The deletion 1a – type sample showed a single T_m at 56°C. The deletion type 1b sample showed a single peak at 47°C.

Table 2. Clinicopathologic data of 102 lung cancer patients

Factors	Exon 19 deletion: Mutation patients (%)	Exon 19 deletion: Wild-type patients (%)	*P*
Mean age (y), 65.5 ± 9.3	7	95	
Stage			
I	3 (42.9)	49 (51.6)	0.9571
II-IV	4 (57.1)	46 (48.4)	
Lymph node metastasis			
N0	3 (42.9)	64 (67.4)	0.3650
N+	4 (57.1)	31 (32.6)	
BI			
≦600	5 (71.4)	35 (36.8)	0.1592
>600	2 (28.6)	60 (63.2)	
Differentiation			
Well	3 (50.0)	35 (47.3)	0.9999
Moderately or poorly	3 (50.0)	39 (52.7)	
Pathologic subtypes			
Adenocarcinoma	4 (57.1)	45 (47.4)	0.9143
Nonadenocarcinoma	3 (42.9)	50 (52.6)	
Age			
≦60	2 (28.6)	26 (27.4)	0.9999
>60	5 (71.4)	69 (72.6)	
Gender			
Male	4 (57.1)	77 (81.1)	0.3051
Female	3 (42.9)	18 (18.9)	

to drug (13). Thus, mutation phenotypes might be correlated with sensitivity for gefitinib therapy. Substitution mutation L858R is located adjacent to the highly conserved DFG motif in the activation motif. The activation loop was known to be important for autoregulation in many kinases (22). For example, the mutation in the activation loop of insulin receptor tyrosine kinase substantially increases the ability of the unphosphorylated kinase to bind ATP (23). From our data, this mutation pattern (L858R) might be more correlated with the populations, such as women, smoking, and adenocarcinoma.

DNA sequencing using the PCR methods described to date is time-consuming and, therefore, may not be suitable for a regular pretherapeutic screening program. Genechip technology is promising but still in its infancy, and adapting this technology to new polymorphisms is time-consuming and expensive. Real-time PCR, on the other hand, allows for easy adoption of new polymorphisms and possibly provides the best means for pretherapeutic genotyping in a clinical setting at present. We, therefore, developed three different PCRs to detect *EGFR* gene mutations and deletions. The advantages of real-time PCR are extensive. The faster PCR method and elimination of additional steps to analyze PCR products save time and minimize the risks of DNA contamination. Handling is facilitated and potentially toxic reagents, such as ethidium bromide stain, are avoided. We have only found 16 of 101 surgically removed samples from Nagoya City University and 6 of 16 gefitinib-treated samples from Kinki-chuo Chest Medical Center. Other mutations might have existed for these patients, although we have only checked the three most frequent mutations. The difference in the ratio of *EGFR* mutation between Nagoya and Kinki patients might have been caused by selection bias because gefitinib was known to be sensitive for female, nonsmoker, and adenocarcinoma patients. Actually, we have checked seven small cell carcinoma and three large cell carcinoma patients from Nagoya and no mutations were found from these patients.

Using the LightCycler reverse transcription-PCR assay described here, the determination of *EGFR* mutation status may be of clinical importance in predicting the sensitivity or resistance to gefitinib therapy for lung cancer. With this method, 32 samples were genotyped within 1 hour without the need of any post-PCR sample manipulation. Mutation detection using real-time PCR with hybridization probes and

Table 3. Genotyping analyses data for the non–small cell lung cancer patients from Kinki-chuo Chest Medical Center

Age	Gender	Mutation	Exon	Mutation type	Pathology	Smoking history
59	F	+	19	del 1a	Adenocarcinoma	N
69	F	+	18	G719S	Adenocarcinoma	N
76	M	+	19	del 1b	Adenocarcinoma	N
56	M	+	19	del 1b	Adenocarcinoma	F/C
33	M	+	19	del 1b	Adenocarcinoma	F/C
59	F	+	21	L858R	Adenocarcinoma	N
47	M	–			Adenocarcinoma	F/C
65	F	–			Adenocarcinoma	N
51	F	–			Adenocarcinoma	N
66	M	–			Adenocarcinoma	F/C
82	M	–			Adenocarcinoma	F/C
71	F	–			BAC	N
66	F	–			BAC	N
71	F	–			Adenocarcinoma	N

Abbreviations: F, female; M, male; del, deletion; BAC, bronchioloalveolar carcinoma; N, never smoker; F/C, former or current smoker.

melting curve analysis can be used for the sensitive detection of DNA mutations. The fast detection of single base substitutions in small amounts of DNA has great potential in pretreated diagnosis and in oncology.

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Oblique approach of computed tomography guided needle biopsy using multiplanar reconstruction image by multidetector-row CT in lung cancer

Tatsuo Kimura [a,*], Nobuyuki Naka [a], Yoshiaki Minato [a], Yasushi Inoue [a], Takeshi Kimura [a], Hidenori Mawatari [a], Setsuko Yamauchi [a], Masanori Akira [b], Masaaki Kawahara [a]

[a] *Department of Internal Medicine, National Kinki Central Hospital for Chest Diseases, 1180 Nagasone-cho, Sakai-City, Osaka 591-8555, Japan*
[b] *Department of Radiology, National Kinki Central Hospital for Chest Diseases, 1180 Nagasone-cho, Sakai-City, Osaka 591-8555, Japan*

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Abstract

The purpose of this study was to establish the technique of multiplanar reconstruction (MPR) with multidetector-row (MDR) computed tomography (CT) guided needle biopsy for the diagnosis to access very difficult lesions. The CT guided percutaneous biopsy are well-established methods to obtain cytological and histological material such as the peripheral tumors in lung cancer. Occasionally, the conventional CT cannot permit planning a trajectory to avoid passage through bones, avoidance of bullae, fissures or vessels. In addition, some lesions are situated in less favorable locations such as those in the costophrenic recess or close to the mediastinum. Rarely can we diagnose them. MPR with MDR-CT has recently become widely available with applications for thoracic lesions. MPR images have been used to evaluate the location of small peripheral lung nodules to the relation of bullaes, vessels, and costophrenic recess. To diagnose these lesions, the usefulness of MPR were evaluated for an planning of an oblique approach of CT guided needle biopsy. MPR images were reconstructed as a line from the needle entry point to the target lesion. The first oblique image applied as the direction of posterior–anterior and cranio-caudal axis, and the second oblique image applied as the direction of posterior–anterior and left–right.

Eleven out of 151 patients were required MPR technique to allow possible access to target, because of avoidance of bone and fissures in the needle pass or located in the costophrenic recess, between April 2001 and December 2002. The 5/11 patients were at the upper site (segment 1, 2 and 6) behind the scapula and ribs, 3/11 patients were at the lower lobe (segment 10) in the costophrenic recess, and 3/11 were middle lobe or segment 3 covered by the ribs and fissures. All the lesions except one were histologically diagnosed. Five patients were adenocarcinoma, and the other five patients were benign tumors. Pneumothorax occurred in one patient before we obtained the specimens. MPR guided needle biopsy with oblique approach was thought to be useful for diagnosis of very difficult thoracic lesions and would obviate an unnecessary surgical thoracoscopy.

Keywords: Multiplanar reconstruction; CT guided needle biopsy; Multidetector-row CT; Lung cancer

1. Introduction

The endoscopical bronchobiopsy and the percutaneous biopsy are today a well-established technique for the diagnosis of lung cancer, and indicate when the histological diagnosis can influence the therapeutic strategy. The endoscopical bronchobiopsy provide the answer to a great extent, however, peripheral tumors not visible on endobronchial examination are diagnosed less readily. The computed tomography (CT) guided percutaneous biopsy is also well-established methods to obtain cytological and histological material such as the peripheral tumors [1–6]. The CT guided biopsy is mainly indicated when the diagnosis cannot be established by bronchoscopic techniques. Percutaneous CT guided biopsy has an overall sensitivity of 70–100% for diagnosis of malignancy, most reports being in the 85–95% range [7]. The most common causes of false-negative are sampling error and inaccurate needle placement [8]. Other cases of false-negative

* Corresponding Author. Present address: Department of Respiratory Medicine, Graduate School of Medicine, Osaka City University, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan. Tel.: +81-6-6645-3801; fax: +81-6-6645-3802.
E-mail address: kimutats@yo.rim.or.jp (T. Kimura).

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are those which CT cannot permit planning a trajectory to avoid passage through bones, avoidance of bullae, fissures or vessels. Occasionally, the lesions are situated in less favorable locations such as those in the costophrenic recess or close to the mediastinum.

The spiral or helical CT imaging, with the ability to obtain a series of continuous images in 30 s during a single breath-hold, has shown promise in the evaluation of complex pathology. Spiral CT creates accurate volume data that can be viewed not only as trans-axial images, but also as multiplanar reconstructions (MPRs) (e.g. coronal, sagittal, or other user-defined oblique planes) [9–11]. Multidetector row (MDR) CT scanner allows for unprecedented speed for CT image acquisition. This acquired speed allows us depiction of the smaller volume of data on the oblique images. Furthermore, the use of MDR-CT significantly clarified the relation of lung tumor to vessels, bullae and pleura [11–13]. These thinner images can be reformatted into off-axis planes to produce unique anatomic displays that were not previously possible. To my knowledge, the idea of the oblique approach to lesions was described as early as 1976 by Greene [14]. However, the conventional CT scan needs a long time to describe the oblique.

The purpose of this study was to establish the oblique approach technique of MPR with MDR-CT guided needle biopsy for the diagnosis to access very difficult lesions. Those accesses may avoid bones, bullae, fissures and vessels, or may be able to access lesions located in the costophrenic recess or close to the mediastinum. These technical improvements of guidance may decrease the rate of biopsies technically impracticable, the rate of complications.

2. Materials and methods

All lesions were imaged on a CT scanner at a dose of 120 kVp, 100 mA. The CT scanner's (LightSpeed Plus; GE Medical Systems, Milwaukee, USA) detector configuration was 2.5 times 4.0 mm in the interspaced high-speed mode, in which four interspaced helical data sets are collected from four 1.25 mm detector rows. The high-speed mode is equivalent to pitch of 3, with the table speed set at 7.5 mm rotation. One rotation of the X-ray tube was 0.5 s. The MPR images were reconstructed and displayed from the transverse images of 15–20 sections (2.5 mm thick). All biopsies were performed with a 18-guage, needle length of 100 mm introducer needle (Hakko, Tokyo, Japan) and a 20-guage, needle length 160 mm core tissue biopsy needle (Bard, Covington, USA) to be used with Bard Magnum biopsy instrument (Bard, Covington, USA).

Oncologists and surgeons at clinical conferences primarily referred patients and the informed consent was obtained. Patients with a unique functional lung, severe cough, severe chronic obstructive pulmonary disease, cardiac insufficiency, or any other contradictions which were considered to be impossible to perform the biopsy, were excluded. Before the first CT scanning, a wire was placed on the skin as a marker on CT images. When the lesions are located in the costophrenic recess or close to the mediastinum, or any trajectory cannot avoid scapula, rib, bullae, or vessel on either breath exhalation or inhalation hold on axial scanning image, MPR images were constituted in order to select the favorable needle entry point. MPR images were reconstructed as a line from the favorable needle entry point to the target lesion. The first oblique image applied as the direction of posterior–anterior axis and cranio-caudal axis, and the second oblique image applied as the direction of posterior–anterior axis and left–right axis. The distances from the needle entry point to the lesion and pleura and the target, and the angle from cranio-caudal axis to considerable needle pass line was calculated (Fig. 1A) on the first oblique image. The distance from the needle entry point to the wire and the angle from left–right axis to needle passing line (Fig. 1B) on the second oblique image. Figs. 1–3 showed the case that the tumor was located at left upper lung at segments 1 + 2. This tumor was located just behind the rib, and faced on bullae. The patient was in prone position. Lesion size was measured along the maximum times minimum diameters. After the favorable needle entry point was marked on the skin, lidocaine 1% solution was used for local anesthesia.

Another two doctors were standing with a protractor at the side of and in front of the patient. They instructed the operator to adjust the direction of needle through the protractor. The guide needle was advanced through the skin to the margin of pleura with the patient's breathing suspended. The first oblique image applied as the direction of posterior–anterior axis and cranio-caudal axis (Fig. 2A). The second oblique

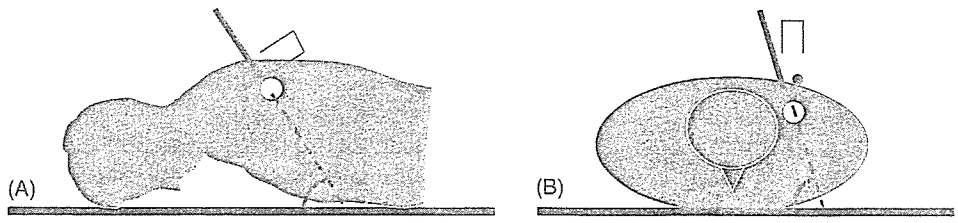

Fig. 1. The schemas of the body position, target lesion and needle direction. The white circle means the target, the black circle means the wire, and the line shows the direction of the needle. Panel A shows the distance from the needle entry point to the lesion and pleura, and the angle from cranio-caudal axis to considerable needle pass line. Panel B shows the distance from the needle entry point to the wire and the angle from left–right axis to needle passing line.

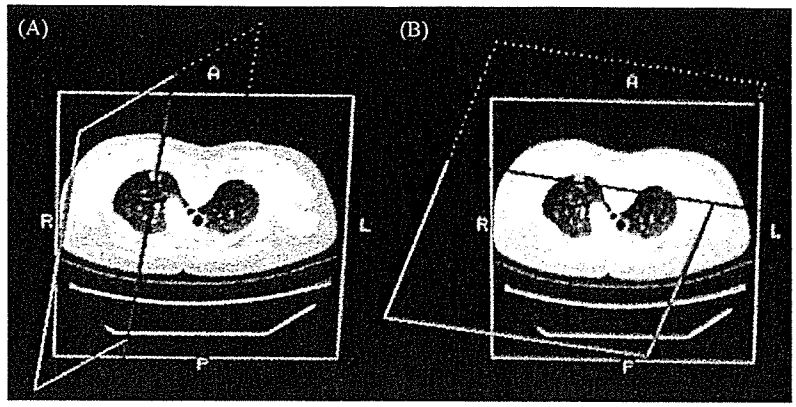

Fig. 2. (A) Induction image of a first oblique image applied as the plane of posterior–anterior axis and cranio-caudal axis. (B) Induction of a second oblique image reconstructed the plane of posterior–anterior and left–right axis.

image applied as the direction of posterior–anterior axis and left–right axis (Fig. 2B), A confirmatory helical acquisition was performed through the needle shaft to the target, and reconstructed into two MPR images. Fig. 3A showed the first oblique images. This image indicated the planes of MPR images corresponding to Fig. 2A. Fig. 3B as the second oblique image, corresponding to Fig. 2B. The inducer needle was adjusted by following the directions of another two doctors with protractors. The CT scanning time was about 30 s and the construction time of the MPR image was about 30 s.

The following technique was almost same as previously reported of CT guided core biopsy [1–3,5,15].

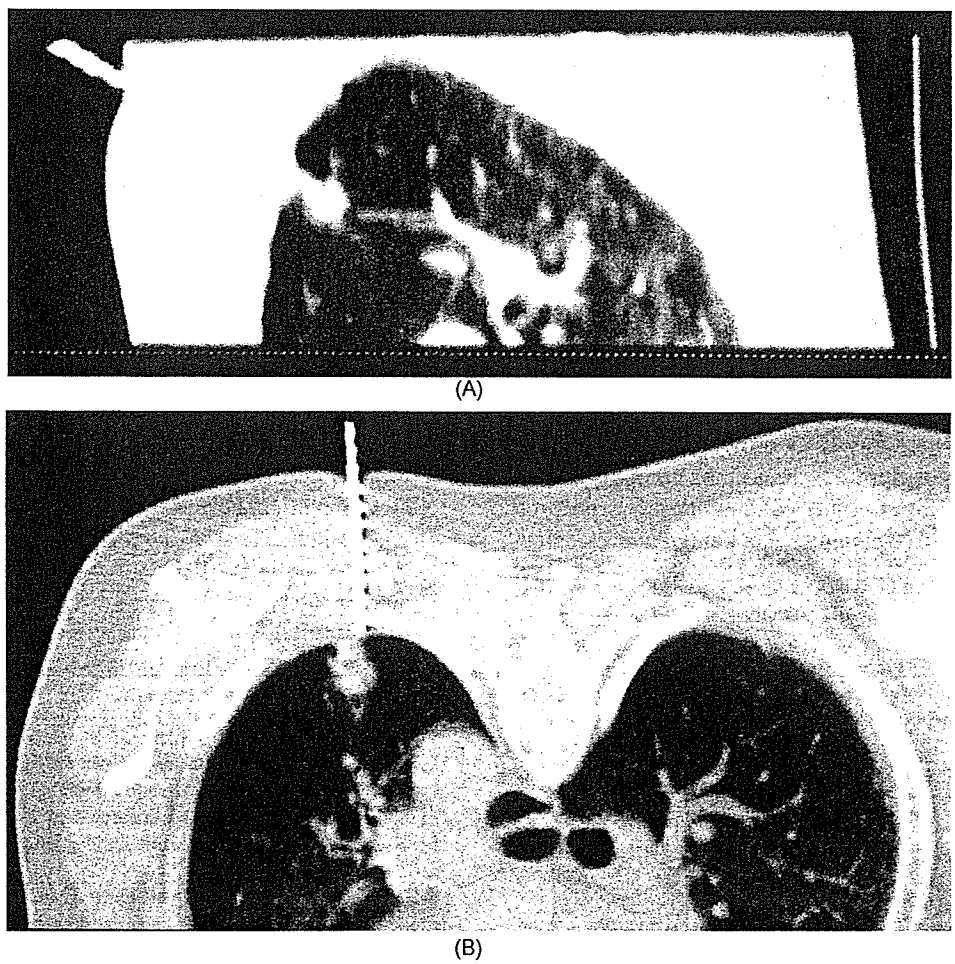

Fig. 3. (A) The first oblique image corresponding to the plane indicated Fig. 2A. (B) The second oblique image corresponding to the plane indicated Fig. 2B.

Briefly, Adjustments in needle position were then made and rechecked until the guide needle tip was immediately adjacent to the proximal edge of the lesion. The core tissue biopsy needle was inserted through the introducer needle and the biopsy system was fired. With a probable tumor, the biopsy was repeated until at least two samples were obtained. The specimens were placed into formalin solution until histological examination. With a suspected infectious illness, the needles were washed by saline for bacteriologic analysis. Each procedure was performed very carefully and one patient needs 30–60 min to finish all steps.

After removal of the biopsy needle, patients were placed in a puncture side-down position for 2 h. All patients had limited CT scans after biopsy to evaluate for the presence of a pneumothorax, when a severe pneumothorax was present, the patients were treated with placement of a chest tube. The next morning, all patients were examined by chest radiographs. Patients with enlarging pneumothoraces on serial chest radiographs or with symptomatic pneumothoraces were treated in the same way.

3. Results

From April 2001 to December 2002, there were 151 patients who underwent CT guided percutaneous lung biopsies at our institution. It was necessary in 11 out of 151 patients to use the MPR technique to access to target, with avoidance of bone and fissures in the needle pass. Table 1 summarizes the patient characteristics including gender, age, the lesion size, the distance from skin to lesion, the segment of each lesion, the patient position, diagnosis and complications. The 5/11 patients, including the case shown previously, were at the upper site (segments 1, 2 and 6) behind the scapula and ribs, 3/11 patients were at the lower lobe (segment 10) in the costophrenic recess, and 3/11 were middle lobe or segment 3 covered by the ribs and fissures. The biopsy was performed with 9/11 patients in prone position and 2/11 patients in supine position. All the lesions except one were histologically diagnosed. Five patients were adenocarcinoma, and the other five patients were benign tumors. Pneumothorax occurred in one patient before we obtained the specimens.

4. Discussion

Lung cancer continues to be the leading cause of cancer death in Japan and the US, with a chance of cure in a minor proportion of patients resected at an early stage. The theoretical advantage of CT for lung cancer screening is its ability to demonstrate small cancers, presumably at stage I [16,17]. The primary tumor size, stage, and were significant prognostic factors for survival [18,19].

Research at Mayo Clinic compared chest radiographs and sputum cytology every 4 months for 6 years against standard follow-up [17]. No overall reduction in mortality, but better survival for individuals in the intervention arm was found. Moreover, that report indicates that CT can be used for screening for lung cancer. Another study of The Early Lung Cancer Action Project group reported the usefulness of annual helical low-dose CT scanning compared with chest radiography in heavy smokers over the age of 60 years [16]. On low-dose CT, they detected small non-calcified nodules of lung cancer at an earlier stage, which are more curable. These kinds of screening are becoming more popular now. As it turned out, the population of tumors situated in the less favorable locations will be increasing. Preoperative diagnosis of a pulmonary nodule by CT biopsy would be necessary for them. The success of diagnosis by CT biopsy would obviate an unnecessary surgical thoracoscopy.

The real-time CT (CT fluoroscopy) was developed to overcome the limitations of conventional CT [20–22]. The methods of guided needle biopsies of the lung using the real-time CT (CT fluoroscopy) can allow real-time visualization of the needle tip or the site of the lesion. In addition, the total time is very short. Compared with CT fluoroscopy, the advantages of MPR guidance of lung biopsies include the following; our methods permit planning a trajectory with avoidance of bullae, fissures or vessels in oblique images. That approach to the lesion might be possible to select from any entry point from any direction. The radiation exposure to the operator

Table 1
Patient characteristics

Number	Gender	Age (years)	Size (mm)	Distance (mm)	Location	Position	Diagnosis	Complication
1	F	71	12 × 12	72	LtS1+2	Prone	Adenocarcinoma	None
2	F	69	20 × 10	52	LtS10	Prone	Adenocarcinoma	None
3	M	47	15 × 10	50	LtS1+2	Prone	Adenocarcinoma	None
4	F	75	20 × 10	16	LtS3	Supine	Adenocarcinoma	None
5	F	60	10 × 10	30	RtS6	Prone	Eosinophilic granuloma	None
6	M	72	30 × 30	62	LtS1+2	Prone	Silicosis	None
7	M	61	10 × 10	46	RtS10	Prone	Eosinophilic granuloma	None
8	M	54	10 × 7	20	RtS3	Supine	Solitary fibrous tumor	None
9	F	53	30 × 10	41	RtS4	Prone	Adenocarcinoma	None
10	M	53	10 × 10	57	RtS2	Prone	No diagnosis	Pneumothorax
11	M	54	8 × 8	46	RtS10	Prone	Tuberculoma	None

is negligible. In our institute, these MPR images were reconstructed from the transverse images of 15–20 sections (2.5 mm thick). The images were not so sharp, but enough clear to evaluate the needle from pleura and target lesion in short time. In the future, CT fluoroscopy technique will combine the advantage of MPR guidance. Further technical improvements of guidance may decrease the rate of biopsies technically impracticable, the rate of complications.

A major complication of a CT guided lung biopsy is the development of a pneumothorax and bleeding. Pneumothorax has been reported from 0 to 61%, 20% in most recent large series and the rate of pneumothoraces requiring treatment with chest tube varies from 1.6 to 17% [7]. Unfortunately, we had one patient whom we could not obtain the specimen because of the pneumothorax. Essentially, the oblique approach needs longer insertion from needle entry point to pleura than that of perpendicular approach. In the oblique approach, the direction of needle is very important. The wrong direction of needle approach cause unexpected complications such as pneumothorax. In addition, the pulmonary nodules move with respiration. The patient's cooperation is thus indispensable to perform CT guided needle biopsies. Slight movement or unstable breath holding during the biopsy renders the initial localization of the lesion inaccurate, making the needle biopsy more difficult, particularly with small lesions [23]. In this regard, as described by Moore, we also recognize patient cooperation to be one of the most important factors necessary for a successful procedure [4].

Previous studies have reported accuracy for CT guided biopsy is related to the size of lesions [1,2,5,15]. In my knowledge, there were few reports that described the relation to the location of tumor and accuracy, except for those [23,24]. In my opinion, the location may affect the success of the biopsy procedures. A limitation of our study is that it was small population and subject to patient selection bias. However, the goal of our study was to improve the technique of CT guidance and increase accuracy and decrease false-negative cases.

Here we reported just technical aspects in this study, and we proved the possibility of oblique needle biopsy with safety and speedy using MDR-CT. It is concluded from present study that MPR guided lung core biopsy is a method of choice for lesions in difficult positions and thought to be useful in the preoperative assessment. It has widened the scope of lesions in unfavorable locations to be targeted accurately. Future studies should include an increased sample size and compare sensitivity and accuracy to other techniques.

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Screening for lung cancer

Masaaki Kawahara

Purpose of review

With the development of newer forms of technology such as low-dose spiral computed tomography, there has been a resurgent interest in screening for lung cancer. The purpose of this review is to highlight recent advances in screening for lung cancer. Articles published since September 2002 are reviewed here.

Recent findings

More frequent screenings (every 4 or 6 months) showed increased mortality from lung cancer, compared with annual screening. A mass screening conducted in 1990 was effective in a case-control study. The results of lung cancer screening by low-dose spiral computed tomography were reported from the Milan group and the Mayo Clinic. Computed tomography depicted peripheral early lung cancer, especially adenocarcinoma. These results are consistent with previous reports from other groups. Screening with imaging becomes more sensitive with automated computerized methods.

Summary

A high percentage of stage IA lung cancers were detected by screening with low-dose helical computed tomography. The characteristics of the nodules detected by low-dose spiral computed tomography have been clarified. There have been many controversial discussions about cost effectiveness and overdiagnosis. There is still no evidence that screening tests reduce the rate of cancer-specific mortality. Several studies of screening for lung cancer are under way.

Keywords

low-dose computed tomography, lung cancer, screening, overdiagnosis, early stage

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Department of Internal Medicine, National Kinki Central Hospital for Chest Disease, Osaka, Japan

Correspondence to Masaaki Kawahara, National Kinki Central Hospital for Chest Diseases, 1180 Nagasone, Sakai, Osaka, 591-8555, Japan
Tel: +81 72 252 3021; fax: +81 72 250 4034;
e-mail: kawaharam@kch.hosp.go.jp

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Abbreviations

ELCAP	Early Lung Cancer Action Project
FDG	fluorodeoxyglucose
PET	positron emission tomography

1040-8746

Introduction

Lung cancer is the leading cause of death from cancer in many industrialized countries [1]. The overall 6-year survival rates remain at approximately 15%, and most patients at diagnosis have advanced disease.

The individual risk of lung cancer depends almost exclusively on exposure to inhaled carcinogens such as cigarette smoke. Therefore, primary prevention of lung cancer is to reduce the exposure to these carcinogens. Secondary prevention aims at diagnosis of preclinical or early stages of lung cancer, particularly non–small-cell lung cancer. Early detection is less efficient and more costly than primary prevention but can be made available for people who have already been exposed to carcinogens. Success in screening for cancer depends on several basic assumptions: there must be effective treatment at the preclinical stage that can reduce mortality in the screened group as compared with the unscreened group. The prevalence, specificity, sensitivity, accessibility, cost, and associated morbidity of the screening method must also be reasonable.

Previous studies using chest radiography and sputum cytology failed to reduce disease-specific mortality [2–5]. This review of screening for lung cancer is based mainly on data published since September 2003.

Screening with sputum cytology and chest radiography

Manser *et al.* [6••] reported a first systemic review of controlled trials to determine whether screening for lung cancer using sputum examination or chest radiography or CT reduces lung cancer mortality. This review included 245,610 subjects. More frequent screening (every 4 or 6 months) (RR 1.11, 95% CI 1.00–1.23) showed increased mortality from lung cancer compared with less frequent screening. In general, the harm associated with screening was poorly reported. Recently used spiral CT has not been incorporated in this study.

Sagawa *et al.* [7•] reported that the smoking adjusted odds ratio for those screened by sputum cytology and chest radiography *versus* those screened by chest radiography only was 0.63, but not significant. They also re-evaluated the efficacy of mass screening for lung cancer done in the 1990s [8•,9–12]. In a matched case-control study, the smoking-adjusted odds ratio in screened persons *versus* nonscreened persons within 12 months of pooled analysis was 0.56 (95% CI 0.48–0.65) with signifi-

cance. However, those authors admitted the existence of some confounding factors. All screening case-control studies are fraught with bias, and this is no exception [13•]. We must await an ongoing prostate, lung, colorectal, and ovarian trial funded by the National Cancer Institute and designed to evaluate the impact of annual chest radiography screening on lung cancer mortality [14].

Screening by use of computed tomography with or without positron emission tomography

Studies using low-dose CT for screening suggest that lung cancer can be detected at an earlier stage and with higher sensitivity than with chest radiography [15–17]. The fact that almost all screen-detected lung cancers were stage I and were successfully resectable led investigators at the Early Lung Cancer Action Project (ELCAP) to cast doubt on the necessity of randomized studies to establish the survival benefit of this screening approach [18]. Swensen *et al.* [19••] reported the results of the Mayo Clinic experience through 2001, including the results from the baseline prevalence and first two annual (incidence) CT examinations in 1520 participants aged 50 years or older who had smoked 20 pack-years or more. Two years after baseline CT screening, 40 cases of lung cancers were diagnosed: 26 at prevalence CT examination and 10 at subsequent incidence CT examination. CT alone depicted 36 cases; 93% of the lung cancer were stage I. Lately, those authors have identified 56 lung cancers (29 prevalence, 23 incidence, and 4 interval [20]. These results are consistent with those of the study by Sobue *et al.* [21] in which 36 lung cancers (14 prevalence, 22 incidence) were found in 1611 participants. In this Anti-Lung Cancer Association project, participants were invited to repeat the same screening twice a year.

The 2-year results of a screening trial for lung cancer in 1035 heavy smokers were reported by Pastorino *et al.* [22••]. These workers of the Milan group used low-dose CT to detect small pulmonary nodules and a diagnostic algorithm, including positron emission tomography (PET) and contrast-enhanced CT, to classify nodules as most likely benign or malignant. They reported a prevalence of 1.1% (11 cancers) and an incidence of 1.1% (11 cancers after 12 months). This study aimed to diagnose malignancy faster by including PET in the diagnostic algorithm. This study adds an important aspect to the field of lung cancer screening with low-dose CT: simplification of the diagnostic algorithm for nodule classification. More data are required to define the ideal algorithm.

In any study, the rate of detection of benign nodules is still high. The selection of the optimal target population is very important. Van Klaveren *et al.* [23••] recommend the inclusion of current smokers or ex-smokers (<5 years) with a smoking history of at least 30 years and an average consumption of at least 20 cigarettes a day.

As an ongoing trial, the National Cancer Institute [24] has launched a study to determine whether screening current and former smokers with spiral CT or chest radiography reduces their risk of dying of lung cancer. This study, called the National Lung Cancer Screening Trial, will enroll 50,000 persons aged 55 to 74 years at 30 sites throughout the United States. Patients in both screening groups will be screened once a year for 3 years, and all participants will be monitored until 2009. To look for biomarkers for early detection of lung cancer, the University of Colorado Specialized Program of Research Excellence (SPORE trial) conducted a cohort study of subjects at high risk for lung cancer (smoking history of ≥30 pack-years and chronic obstructive pulmonary disease defined by spirometry) [25]. McWilliams *et al.* conducted a pilot study that used the combined techniques of automated quantitative image cytometry (AQC) of sputum cells, autofluorescence bronchoscopy, and spiral CT [26]. AQC improved the detection rate of lung cancer from 1.8 to 3.1%.

Pulmonary nodules

Karabulut *et al.* [27] compared low-dose CT with standard CT in the evaluation of pulmonary nodules. This comparison was prospectively done in the same patients. There were no statistically significant differences in the number of nodules detected at standard CT or low-dose CT.

Li *et al.* [28] studied the differences in the appearance of the cancers in nonsmokers *versus* smokers in Japan. Most of the lung cancers in nonsmokers were slow-growing adenocarcinomas appearing as faint ground-glass opacities on CT, whereas rapidly growing cancers appearing as solid nodules were more commonly seen in smokers.

The detection rate for lung cancer was 1.1% for both nonsmokers (45 of 4,251) and smokers (39 of 3596). The prevalence of well-differentiated adenocarcinomas was greater in nonsmokers (88%, 22 of 25) than in smokers (29%, 4 of 14) ($P < 0.001$). The prevalence and incidence of pathologic stage IA disease were greater in nonsmokers than in smokers (92% [22 of 24] *vs* 58% [7 of 12] and 100% [19 of 19] *vs* 70% [14 of 20], respectively) (both $P < 0.05$). The mean size of the tumors in the nonsmokers (12.4 mm) was smaller than in smokers (18.2 mm) ($P < 0.001$). The percentage of cancers categorized as pure or mixed ground-glass opacity (86%, 38 of 44) on CT was greater in nonsmokers than in smokers (46%, 16 of 35) ($P < 0.001$). The authors included nonsmokers as well as smokers in their screening. Henschke *et al.* [29] reported that the malignancy rate was significantly higher for part-solid nodules than for either solid ($P = 0.004$) or nonsolid nodules ($P = 0.03$). The malignancy

type in the part-solid or nonsolid nodules was predominantly bronchioloalveolar carcinoma or adenocarcinoma with bronchioloalveolar features, contrasting with other subtypes of adenocarcinoma found in the solid nodules ($P = 0.0001$). At annual repeat screenings, only 30 instances of positive test results have been obtained; 7 of these involved part-solid or nonsolid nodules. The morphology of the nodules needs to be further classified.

Armato *et al.* [30] evaluated the performance of a fully automated computerized method for the detection of lung nodules in CT scans in the identification of lung cancers that may be missed during visual interpretation. Using this method, Armato *et al.* [32] reported that a large fraction of missed cancers (84%, 32 of 38) in a database of low-dose CT scans were detected correctly. This may help reduce the burden on the visual interpreter.

Aoyama *et al.* [32] reported that the automated method helped radiologists eliminate many benign nodules in a lung cancer screening program with low-dose CT. With a large base of 489 nodules, the performance of the automated computerized scheme with multiple slices of nodule images for determination of the likelihood measure of malignancy was greater than that with a single slice of nodule images. There was an improvement in distinguishing benign from malignant nodules when this method was used, compared with the results obtained by radiologists alone.

Ford *et al.* [33] reported on the adherence of screening. Statistically significant predictors of nonadherence by multivariate results were false positive cases with current or past smoking status. Additional predictors were being African American ($P < 0.01$), being female ($P < 0.001$), and having a high school education or less ($P < 0.01$). False positive results had a stronger effect on nonadherence among ever-smokers than among never-smokers.

Overdiagnosis represents a subclinical condition that would not have produced signs or symptoms before the individual died of other causes [34]. It may cause the person being screened to worry for months or years about having cancer.

Yankelevitz *et al.* [35] calculated the doubling times of stage I cancers detected by the Mayo Lung Project (MLP) and Memorial Sloan-Kettering Cancer Center (MSK) to estimate the frequency of overdiagnosis. The median doubling times were 101 days in the MLP and 144 days in the MSK. Only 5% had doubling times exceeding 400 days; 10% exceeded 300 days. The ELCAP group contradicted the idea that screening in the MLP with chest radiography led to a high proportion of overdiagnosis among diagnoses of early-stage lung carcinoma [36].

Kashiwabara *et al.* [37] evaluated the outcome in 45 patients with lung cancer found on lung cancer mass screening roentgenograms, but who did not subsequently consult a doctor. A 1-year delay in treatment itself affected the outcome. In their study, the tumor sizes in the delayed consultation group were 10 to 20 mm in 4 patients and greater than 20 mm in 41 patients, and there were no patients with tumor sizes less than 10 mm. This may not serve as a reference of small nodules less than 10 mm tumor.

Li *et al.* [38•] also showed that lung cancers were missed at low-dose CT screening in a general population in Nagano, Japan. All missed cancers were intrapulmonary, and 28 (88%) were stage IA. All 20 detection errors occurred in cases of adenocarcinoma, 17 (85%) of which were well-differentiated tumors and 11 (55%) of which were in nonsmoking women. These lung cancers were very subtle and appeared as small faint nodules, overlapping normal structures, or opacities in a complex background of other disease such as tuberculosis, emphysema, or lung fibrosis. This was the first study on characteristics of lung cancers missed at CT screening in a general population, including nonsmokers and women.

Takashima *et al.* [39] in Nagano, Japan, showed the reliability of high-resolution CT features of benign lesions, which were small solitary pulmonary nodules (≤1 cm) detected by population-based CT screening for lung cancer. Takashima *et al.* [40] advocated the usefulness of follow-up CT with a combination of findings on initial and follow-up CT to differentiate benign and malignant nodules.

A serious concern has been raised that the better our methods of detection become, the more overdiagnosis of lung cancer we will have. Ost *et al.* [41] briefly reviewed the clinical problem with the solitary pulmonary nodule.

Cost-effectiveness study

Lung cancer screening with low-dose CT is likely to be cost effective if the screening process can detect more than 50% of cancers at a localized stage [42].

Preliminary results of baseline screening were released by Wisnivesky *et al.* [43]. Data from the ELCAP were incorporated into a decision analysis model comparing low-dose CT scan screening of high-risk individuals (*ie* those ≥60 years old with at least 10 pack-years of cigarette smoking and no other malignancies) to observation without screening. The incremental cost-effectiveness ratio of a single baseline low-dose CT scan was $2500 per year of life saved. In the base-case analysis, screening would be expected to increase survival by 0.1 year at an incremental cost of approximately $230. The authors concluded that a baseline low-dose CT scan for lung cancer screening is potentially highly cost-effective.

By contrast, Mahadevia *et al.* [44••] estimated the potential benefits, harms, and cost-effectiveness of lung cancer screening with helical CT in various efficacy scenarios. They compared annual helical CT screening with no screening for hypothetical cohorts of 100,000 current, quitting, and former heavy smokers, aged 60 years, of whom 55% were men. In multiway sensitivity analyses, a program screening current smokers was $42,500 per quality adjusted life years (QALY) gained if extremely favorable estimates were used for all of the influential parameters simultaneously. The authors concluded that given the current uncertainty of benefits, the harms from invasive testing, and the high costs associated with screening, direct-to-consumer marketing of helical CT is not advisable. Future advancements in lung cancer diagnosis and treatment could make their result out of date.

Comber *et al.* [45]. studied the impact of quantitative contrast-enhanced CT (QECT) on the cost-effectiveness of fluorodeoxyglucose (FDG)-PET. The QECT strategy incurred the least cost ($5560/patient), but the QECT+PET strategy was the most cost effective (incremental cost-to-accuracy ratio $12059/patient). The problem was the low specificity of QECT: they assumed it to be 0.58. This technique awaits further validation.

Centrally located lung cancer

The sensitivity of bronchoscopic detection of early lung cancer depends on the size of the nodule, the site of the lesion, and the prevalence in the study population.

The focus of screening seems to have moved toward peripheral lung cancer. However, detection of centrally located lung cancer is still important. Recent developments in the detection of preinvasive lesions of the large airways by fluorescence bronchoscopy have been reviewed by Banerjee *et al.* [46•]. Sutedja [47•] recently reviewed new techniques such as fluorescence bronchoscopy and innovative sputum screening.

Effect of screening on smoking habit

Schnoll *et al.* [48] reported psychologic issues related to the use of spiral CT. Greater motivation of female smokers to quit smoking was related to greater age, lower nicotine addiction, fewer health symptoms, and higher quitting self-efficacy and pros of quitting. In their study, 16% of enrollees quit smoking after screening. Schnoll *et al.* [49] also showed that 59% of smokers were interested in smoking cessation counseling, with screening.

Several excellent reviews, comments, or editorials on the screening for lung cancer have been published recently [50•–58•].

Conclusion

Although low-dose CT can depict early-stage lung cancers, the rate of benign nodule detection is still high. Screening with imaging has become more sophisticated. There is still no evidence that screening tests reduce the rate of cancer-specific mortality. Its efficiency depends on many factors such as the advancement of diagnostic methods, financial cost, and psychologic effect as well as the prevalence of curable lung cancer in the screened population.

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