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The adenocarcinoma-specific stage shift in the Anti-lung Cancer Association project: Significance of repeated screening for lung cancer for more than 5 years with low-dose helical computed tomography in a high-risk cohort☆

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ABSTRACT

Background: We investigated whether a stage shift occurs during long-term repeated screening for lung cancer with low-dose helical computed tomography (LDCT) in a high-risk cohort.
Methods: A total of 2120 subjects (mean age, 63 years; 87% male and 83% smokers) were continuously recruited and underwent repeated screening with LDCT from 1993 through 2004.
Results: Nineteen lung cancers were detected at baseline examinations (prevalence cancers), and 57 lung cancers were detected at subsequent examinations (incidence cancers). For both prevalence cancers and incidence cancers, adenocarcinoma (74% and 63%, respectively), especially invasive adenocarcinoma (42% and 23%, respectively), was the most common histological diagnosis, and stage IA was the most common pathological stage (58% and 79%, respectively). The detection rate of incidence cancers other than bronchioloalveolar carcinoma became significantly higher after 5 years of LDCT examinations ($r = 0.50$, $P = 0.020$). Moreover, both the percentage of cancers of stage II–IV and tumor size became significantly lower for invasive adenocarcinoma after 5 years of LDCT examinations ($r = -0.77$, $P = 0.007$ and $r = -0.60$, $P = 0.029$, respectively).
Conclusions: Repeated screening for more than 5 years might demonstrate the efficacy of LDCT screening for lung cancer through an adenocarcinoma-specific stage shift.

1. Introduction

Lung cancer is considered as an appropriate disease for screening because it is the leading cause of cancer death worldwide, symptomatic disease is generally lethal, localized disease can be managed curatively, and high-risk cohorts can be defined on the basis of tobacco consumption [1]. However, screening with chest X-ray films or sputum cytological examination has failed to reduce lung-cancer mortality rates in randomized, controlled trials [2–6].

Low-dose helical computed tomography (LDCT) is a promising screening method because a higher percentage of asymptomatic, X-ray–invisible, or stage IA lung cancers (mostly adenocarcinoma) are found with baseline or repeated computed tomography (CT) examinations than with conventional screening methods [7–11]. In fact, according to the results of the International Early Lung Cancer Action Program, the 10-year survival rate for all patients with lung cancer was 80% regardless of stage or treatment [12]. If the cancer was in clinical stage I and was promptly resected, the 10-year survival rate was 92%. However, because large, randomized, controlled trials of LDCT screening are still in progress [13,14], whether LDCT screening reduces lung-cancer mortality rates remains uncertain. Although mortality data are needed to determine whether LDCT screening is effective, indirect evidence for a possible mor-

Abbreviations: CT, computed tomography; LDCT, low-dose helical computed tomography; BAC, bronchioloalveolar cell carcinoma; ALCA, Anti-lung Cancer Association.

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tality reduction can be obtained from a "stage shift," an increase in the detection rate of putatively curable early-stage lung cancers and a concomitant decrease in incurable late-stage cancers, leading to a decrease in the lung-cancer-specific mortality rate [15], which can be used as a surrogate endpoint even in a nonrandomized, uncontrolled trial.

Results of many single-armed, uncontrolled trials of annual screening with LDCT have been published [12,16–22]. However, none of these trials has documented a stage shift, perhaps because the number of lung cancers detected with repeated screening was too small (range, 4–35 cancers) or because the duration of repeated screening (range, 1–4 years) was too short. Thus, to determine whether a true stage shift occurs, a longer-term LDCT study with a larger number of detected lung cancers is required.

Furthermore, studies performed to date have not considered the effect of histological classification on the stage shift. Recent LDCT trials suggest that an increase in early-stage lung cancer might not be accompanied by a decrease in late-stage lung cancer (i.e., overdiagnosis) [15] and that the presence of localized bronchioloalveolar cell carcinoma (BAC) and mixed adenocarcinoma with BAC component might reflect overdiagnosis bias, although adenocarcinoma without BAC component behaves as aggressively as do other non-small cell carcinomas [23].

In the present study, on the basis of an update of the Anti-lung Cancer Association (ALCA) project [16], we investigated whether a stage shift occurs when lung cancers are stratified by histological subtype during long-term repeated LDCT screening for lung cancer in a high-risk cohort comprising mostly male smokers in their 60s.

2. Patients and methods

2.1. Study population

From September 1993 through August 2004, LDCT screening was performed semiannually by the ALCA in Tokyo. The ALCA is a for-profit organization established in 1975 to thoroughly screen for lung cancer in dues-paying participants. Because the participants are continuously recruited from members of the general population 40 years or older with a history of smoking (>20 pack-years) or a single episode of hemoptysis within the past 6 months, most participants are male smokers in their 60s. Written informed consent was obtained from each participant at baseline CT screening.

2.2. Screening procedures

Screening was performed as described previously [16]. Briefly, at baseline screening a simple questionnaire about smoking history and symptoms was completed, and LDCT, chest radiography (posterior–anterior position), and sputum cytological examination pooled for 3 days were performed. Participants were invited twice a year by mail after the baseline screening to repeat the same screening procedures. The CT scanner (TCT-900S Superhelix, Toshiba Medical, Tokyo, Japan) was used under the following conditions: 120 kVp, 50 mA, 10-mm collimation, 1 rotation of the X-ray tube per second, and a table speed of 20 mm/s (pitch, 2:1). Image construction was performed with 180° linear interpolation at 1-cm intervals. All CT images were examined by 2 of 7 readers (radiologists or thoracic physicians).

2.3. Evaluation of detected lung cancers

The staging and the histological classification of detected lung cancers were performed according to the International System for Staging Lung Cancer [24] and the World Health Organization lung tumor classification system [25], respectively. Cancers were classified as adenocarcinoma, squamous cell carcinoma, other non-small cell carcinoma, or small cell carcinoma. Moreover, adenocarcinoma was subclassified on the basis of the histological growth pattern as localized BAC, mixed adenocarcinoma with BAC component, and adenocarcinoma without BAC component (invasive adenocarcinoma).

Lung cancers detected at baseline screening were considered "prevalence cancers," whereas those newly detected at subsequent repeated LDCT screening examinations were considered "incidence cancers." Furthermore, lung cancers diagnosed outside our semiannual LDCT screening procedure within a screening interval were defined as "interval cancers," whereas those diagnosed outside our screening procedure after a period longer than the screening interval (due to refusal by ALCA participants) were not classified as "interval cancers." The presence or absence of interval cancers was confirmed through questionnaire when participants were invited twice a year by mail after the baseline screening to repeat the same screening procedures.

Excluded from analysis were 6 cases of hilar lung cancer detected on sputum cytological examinations or on evaluation of hemoptysis but not with LDCT.

2.4. Statistical analysis

Statistical P values for the differences in percentages and means were evaluated with the χ^2 test and the t-test, respectively. Survival curves were estimated with the Kaplan–Meier method, with survival time defined as starting from when microscopic evidence for malignancy was first obtained to the date of death or November 25, 2005, whichever came first. Differences in survival rates between groups were evaluated with the log-rank test. Multivariate Cox proportional hazards model analysis was performed to identify significantly independent prognostic factors for overall survival. Linear regression analysis with the least-squares method was performed for the relationships between groups. All calculations were performed with Stat View 5.0J software (SAS Institute Inc., Cary, NC). P values less than 0.05 were considered to indicate statistical significance.

3. Results

3.1. Characteristics of participants

During the study period, 20,113 LDCT scans were performed for 2120 ALCA participants (mean age, 63 years; 87% male and 83% smokers), and 76 peripheral lung cancers were detected. Participants underwent LDCT screening a median number of 7 times (range, 1–22 times; Fig. 1A); a median number of 3 lung cancers were detected in each ordinal screening (range 0–9; Fig. 1B); a median of 3.5 years had passed since a participant's baseline screening (range, 0–10.5; Fig. 1C); and a median of 0.5 years had passed since a participant's previous screening (range, 0–10.0; Fig. 1D). Of the 2120 ALCA participants, 243 (11%) underwent only baseline LDCT screening, 753 (36%) underwent repeated LDCT screening for more than 5 years, and 322 (15%) underwent repeated LDCT screening for more than 10 years.

3.2. Comparison of results between baseline and subsequent LDCT screenings

The characteristics of all participants and of participants who underwent at least 1 subsequent LDCT screening examination are shown in Table 1. No significant difference was observed between these groups in terms of age, sex, or smoking status at baseline. However, the detection rate of lung cancer was significantly higher

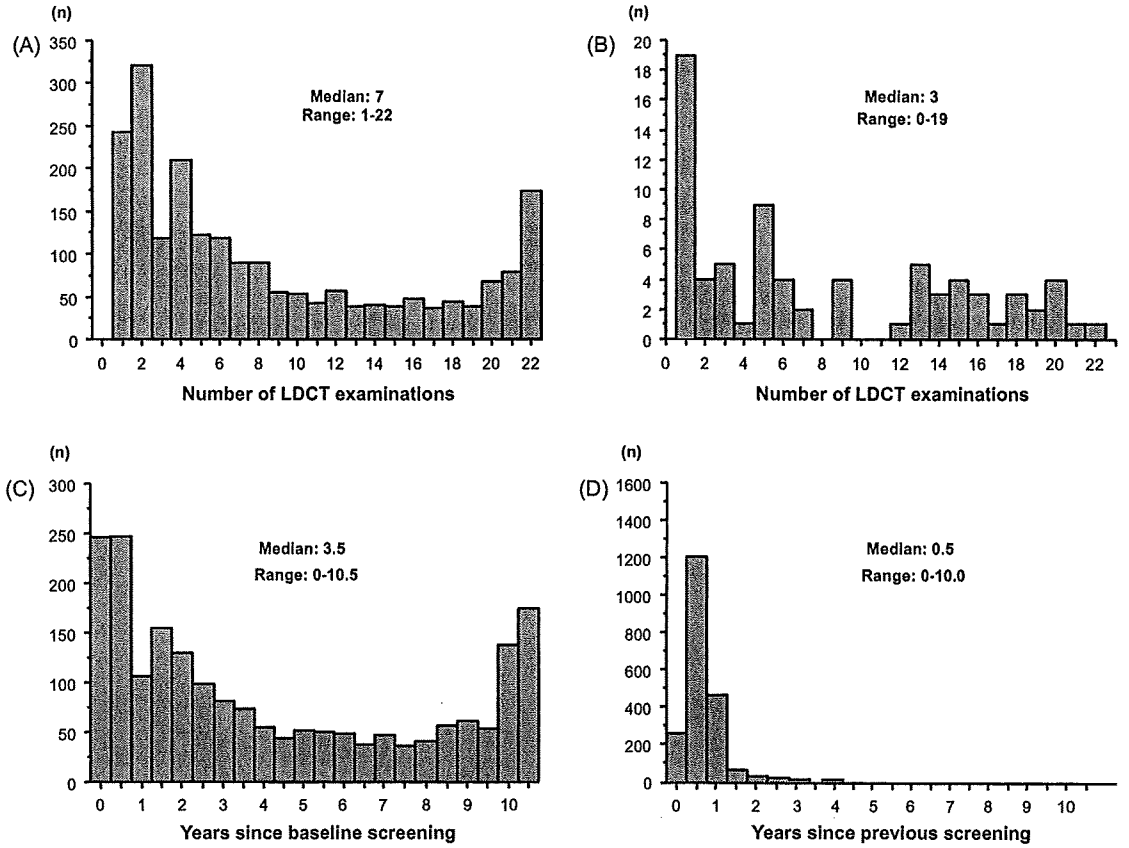

Fig. 1. Characteristics of repeated LDCT screening. (A) Distribution of the number of times participants underwent repeated LDCT screening (*X* axis indicates the number of LDCT examinations, and *Y* axis indicates the number of participants in each ordinal screening). (B) Distribution of the number of lung cancers detected in screening examinations grouped by ordinal number (*X* axis indicates the number of LDCT examinations, and *Y* axis indicates the number of lung cancers detected in each ordinal screening.). (C) Distribution of years since participants had undergone baseline screening (*X* axis indicates years since baseline screening, and *Y* axis indicates the number of participants in each ordinal screening period). (D) Distribution of years since participants had undergone previous screening (*X* axis indicates years since previous screening, and *Y* axis indicates the number of participants in each ordinal year since previous screening).

at baseline screening (0.90%: 19 prevalence cancers in 2120 participants) than at repeated screenings (0.32%: 57 incidence cancers in 1877 participants; $P<0.001$).

The characteristics of 76 patients with lung cancers detected at screening examinations are summarized in Table 2. The 19 patients with prevalence cancers and the 57 patients with incidence cancers did not differ in age, sex, or smoking status. However, both the percentage of positive chest X-ray films (53% vs. 16%, $P=0.004$) and tumor size (24 mm vs. 17 mm, $P=0.018$) were significantly less in patients with incidence cancers than in patients with prevalence cancers. Although neither histological diagnosis nor pathological stage differed significantly between patients with prevalence cancers and those with incidence cancers, in both groups of patients adenocarcinoma (74% and 63%, respectively), especially invasive adenocarcinoma (42% and 23%, respectively), was the most common histological diagnosis and stage IA was the most common pathological stage (58% and 79%, respectively).

Table 1
Characteristics of participants.

	Baseline LDCT	Repeated LDCT	*P*
No. of participants	2120	1877	
Age (years, mean ± SD)[a]	63 ± 11	64 ± 11	NS
Sex (% male)	87	88	NS
Smoking (% smokers)[a]	83	84	NS
No. of detected lung cancers	19	57	
No. of screenings	2120	17993	
Detection rate (%)	0.90	0.32	<0.001

[a] Fixed at baseline screening.

Survival rates were compared between patients with prevalence cancers and those with incidence cancers. The 5- and 10-year survival rates were 84.5% and 84.5%, respectively, in patients with incidence cancers ($n=57$) and were 68.7% and 38.1%, respectively, in

Table 2
Clinicopathological characteristics of patients with screening-detected lung cancer.

	Prevalence cancers	Incidence cancers	*P*
No. of patients	19	57	
Age (years, mean ± SD)[a]	66 ± 8	69 ± 9	NS
Sex (% male)	84	86	NS
Smoking (% smokers)[a]	89	93	NS
Positive X-ray (%)	53	16	0.004
Tumor size (mm, mean ± SD)	24 ± 15	17 ± 10	0.018
Histological type			NS
Adenocarcinoma	14 (74%)	36 (63%)	
BAC	2	11	
Adenocarcinoma with BAC	4	12	
Invasive adenocarcinoma	8	13	
Squamous cell carcinoma	4	12	
Other non-small cell carcinoma	1	5	
Small cell carcinoma	0	4	
Pathological stage			NS
IA	11 (58%)	45 (79%)	
IB	2	3	
II	0	3	
III	5	4	
IV	1	2	

BAC: bronchioloalveolar cell carcinoma.
[a] Fixed at baseline screening.

patients with prevalence cancers ($n = 19$). No significant difference was observed between the groups (log-rank test, $P = 0.208$). Multivariate analysis with the Cox proportional hazards model found that only pathological stage ($P = 0.006$) was an independent prognostic factor for overall survival. The risk of death in patients with stage II–IV disease was increased 8.26-fold (95% confidence interval, 1.85–37.03). In contrast, age, sex, smoking status, tumor size, histological subtype (presence of BAC component), and screening type (baseline vs. repeated) were not independent prognostic factors.

No interval lung cancers were detected outside our semiannual LDCT screening procedure within a screening interval. However, 3 lung cancers were detected outside our screening procedure after a period longer than the screening interval. For these 3 lung cancers, the histological classification and stage, screening period from baseline to previous screening, and time since previous screening, respectively, were: invasive adenocarcinoma, stage IV, 5 years, and 4 years; squamous cell carcinoma, stage IA, 3.5 years, and 5 years; and other non-small cell carcinoma, stage II, 5 years, and 1.5 years.

3.3. The presence of an increased detection rate, a stage shift, and a size shift

The detection rate of all 57 incidence cancers was positively correlated with the duration of repeated screening ($r = 0.50$, $P = 0.020$) but remained uncorrelated if the duration of repeated screening was 5 years or less (Fig. 2A). In contrast, the detection rate of localized BAC showed a weak negative correlation with the duration of repeated screening ($r = -0.38$, $P = 0.086$). Other histological subtypes, including invasive adenocarcinoma, showed no significant correlations.

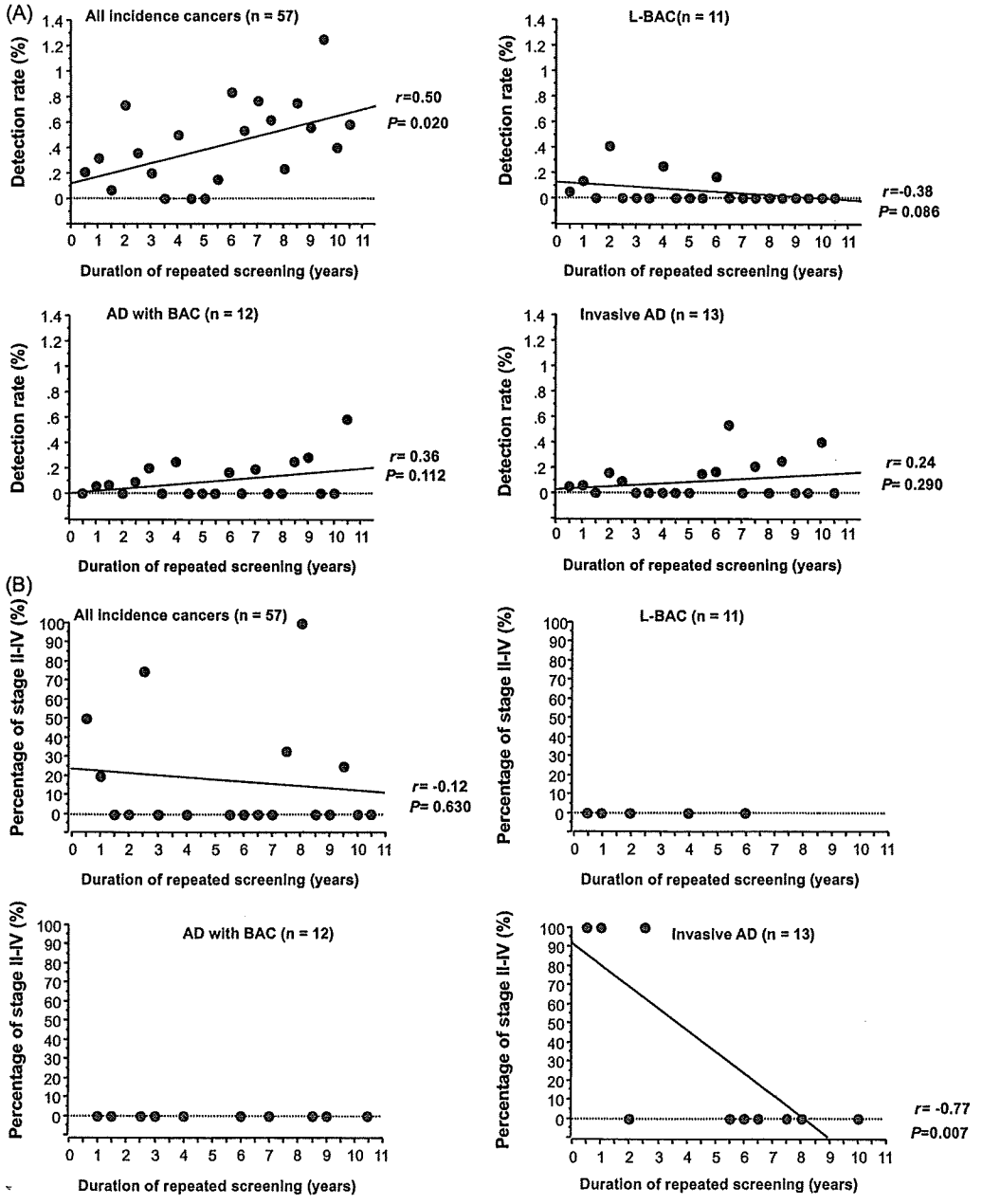

Fig. 2. Relationship between the duration of repeated screening and characteristics of incidence lung cancers. Correlations between the duration of repeated screening and the detection rate (A), the proportion of stage II–IV disease (B), and tumor size (C) were evaluated according to histological subtypes. L-BAC, localized bronchioloalveolar carcinoma; AD, adenocarcinoma.

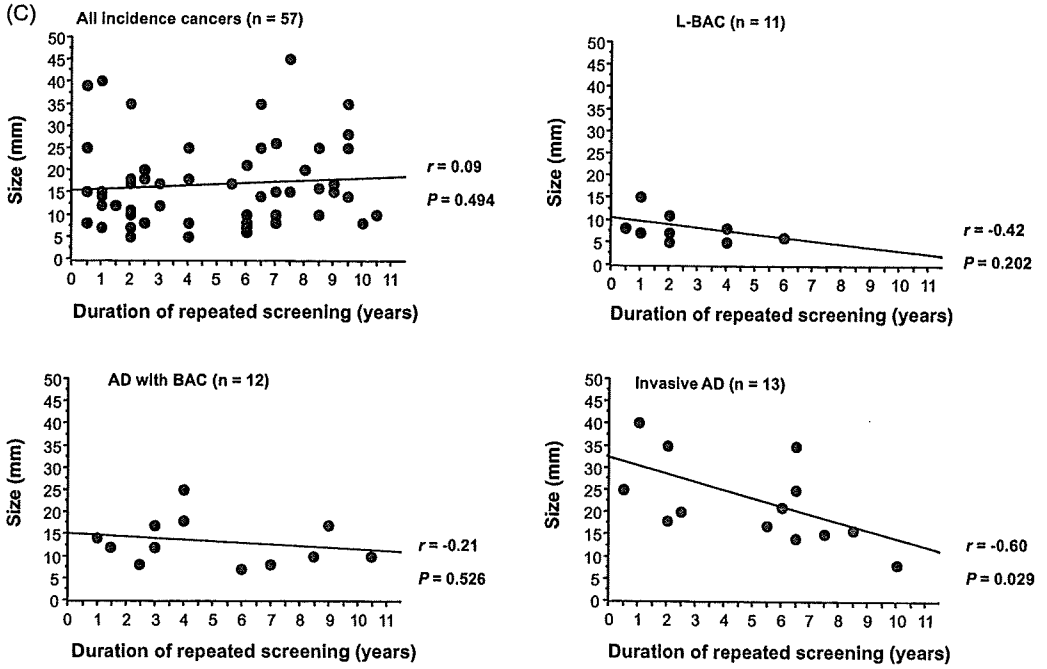

Fig. 2. (*Continued*).

Although the percentage of stage II–IV disease among all 57 incidence cancers was not correlated with the duration of repeated screening ($r=-0.12$, $P=0.630$), the percentage of stage II–IV disease among invasive adenocarcinoma was negatively correlated with the duration of repeated screening ($r=-0.77$, $P=0.007$) but remained uncorrelated if the duration of repeated screening was 5 years or less (Fig. 2B). In contrast, the percentage of stage II–IV disease among both localized BAC and mixed adenocarcinoma with BAC component remained 0% regardless of the duration of repeated screening. Neither squamous cell carcinoma ($r=-0.12$, $P=0.767$) nor small cell carcinoma ($r=-0.67$, $P=0.999$) showed a significant correlation between the percentage of stage II–IV disease and the duration of repeated screening.

Similarly, although tumor size among all 57 incidence cancers was not correlated with the duration of repeated screening ($r=-0.12$, $P=0.630$), the tumor size of invasive adenocarcinoma was negatively correlated with the duration of repeated screening ($r=-0.60$, $P=0.029$) but remained uncorrelated if the duration of repeated screening was 5 years or less (Fig. 2C). In contrast, other histological subtypes showed no significant correlations.

4. Discussion

In the present study involving 10 years of semiannual LDCT screening in a continuously recruited cohort comprising mostly male smokers in their 60s, increased detection rates were observed for lung cancers other than localized BAC. Moreover, both a stage shift and a size shift were observed for invasive adenocarcinoma of the lung. This report is, to our knowledge, the first to document the significance of long-term repeated screening for lung cancer with LDCT in a high-risk cohort.

Recently, Bach et al. have demonstrated that screening for lung cancer with LDCT may not meaningfully reduce the risk of advanced lung cancer or death from lung cancer [26]. Their conclusion was based on a model predicting deaths from lung cancer applied to 3 studies of LDCT screening in asymptomatic population at risk for lung cancer [20–22]. However, most importantly, the screening period of each of the 3 studies was less than 5 years. If each screening period had been 5 years or longer, Bach et al. might have instead confirmed a decrease in the lung-cancer-specific mortality rate. The screening period is important for other cancers for which the efficacy of screening has already been demonstrated; for example, the period of screening with fecal occult blood for colorectal cancer has been shown to be the important factor in a large randomized, controlled trial [27]. The initial protocol of the study specified 5 years of screening; however, the Policy and Data Monitoring Group recommended that screening be reinstituted because of the lack of statistical power regarding the mortality rate through 5 years of screening in the population. Screening then continued for 10 years, resulting in the finding of a lower mortality rate in screened subjects. Furthermore, meta-analysis of 8 randomized, controlled trials of screening mammography has demonstrated a statistically significant reduction in mortality rate among women aged 40–49 years at entry through screening for 10 years [28]. In particular, in 1 of these studies, the mortality rate from breast cancer was similar in screened group and the control group during the first 8 years but then became lower in screened group after 8 years [29]. Therefore, the efficacy of repeated screening for lung cancer might be demonstrated only with a long screening period.

To determine whether LDCT screening can reduce the mortality rate from lung cancer, a large, randomized, controlled trial has been started in the United States (National Lung Screening Trial) [13]. In this trial, 50,000 subjects at high risk for lung cancer were randomly assigned to undergo screening with chest radiography or LDCT at baseline and then annually for 2 additional years with annual telephone follow-up thereafter. Accrual was completed in February 2004, and final analyses are scheduled to be completed in 2009. In addition, a Dutch-Belgian randomized trial (NELSON trial) comparing CT screening with no screening at baseline and then 2 repeated screenings within 3 additional years in almost 20,000 subjects at high risk for lung cancer should be completed by 2010 [14]. However, if only long-term, repeated LDCT screening produces a stage shift, these 2 trials of short-term, repeated LDCT screening might fail to show any benefit. In fact, we should note that the detection rate of incidence lung cancers of all types remained unchanged if the duration of repeated screening was 5 years or less. Furthermore, neither a stage shift nor a size shift in invasive adenocarcinoma occurred if the duration of repeated screening was 5

years or less. Therefore, considering our present findings that the detection rate of incidence lung cancers in a cohort of mostly male smokers increased after 5 years of repeated LDCT screening and that the stage shift was observed for at least invasive adenocarcinoma after long-term, repeated LDCT screening for 5 years, we believe that proving the efficacy of LDCT screening would be difficult if the screening period is less than 5 years.

In the present study both a stage shift and a size shift were observed for invasive adenocarcinoma of the most common histological diagnosis. Considering direct evidence exists for a stage-size relationship in LDCT screen-diagnosed lung cancers [30], the fact that the stage shift was followed by a simultaneous size shift supports the occurrence of a stage shift in invasive adenocarcinoma. However, we wonder why this phenomenon was observed for only invasive adenocarcinoma. This question is difficult to answer, considering that invasive adenocarcinoma behaves as aggressively as do other non-small cell carcinomas. A possible explanation might simply be that the number of incidence lung cancers detected in our study lacks sufficient statistical power. However, some adenocarcinomas have higher volume-doubling times, grow more slowly, and are, therefore, diagnosed more easily at an early stage; another explanation could be length-time-biased sampling inherent to single-armed, uncontrolled trials. Thus, large, randomized, controlled trials on the basis of long-term repeated screening will be necessary to answer this question.

In the present study, we have performed semiannual LDCT screening to detect aggressive, fast-growing lung cancers at an early stage. However, no interval lung cancers were detected in our screening population. On the other hand, an interesting phenomenon is shown by the characteristics of 3 patients with lung cancers detected outside our screening procedure after a period longer than the screening interval. These lung cancers were detected after the patients had stopped undergoing semiannual LDCT screening because no abnormality was observed during the screening periods, which were 3.5 years in 1 patient and 5 years in 2 patients. Therefore, these facts suggest the efficacy of long-term repeated LDCT screening for more than 5 years.

We have several concerns about our study. The first concern is that, in addition to the stage shift caused by long-term repeated screening, we estimated the efficacy of long-term repeated screening could also be shown indirectly if the overall survival of patients with incidence cancers would be significantly longer than that of patients with prevalence cancers. So, we compared baseline screening with subsequent screening. However, multivariate Cox proportional hazard model analysis showed that the screening type (baseline vs. repeated screening) was not an independent prognostic factor for overall survival. A possible reason for this finding is the small number of participants and, therefore, the small number of deaths from lung cancer in both groups. Thus, larger studies involving larger numbers of participants are needed to investigate whether the overall survival of patients with incidence cancers is, in fact, significantly longer than that of patients with prevalence cancers because of the efficacy of long-term repeated screening. A second concern is that the partial-volume effect might affect the ability of screening CT images to demonstrate small nodules because only thick-section screening CT with image construction at 1-cm intervals was available during the screening period. Therefore, in a second ALCA study still in progress we have performed both chest radiography and LDCT to evaluate the detection power of LDCT in terms of the partial-volume effect. A third concern associated with long-term semiannually repeated LDCT screening is that a large number of healthy persons would be exposed to radiation and have an increased risk of radiation-induced lung cancer, although the risk of radiation-induced cancers other than lung cancer would be far lower [31,32]. According to one estimate, LDCT screening at a rate of 1.5 examinations per year would induce 4.5 lung cancers per year in 100,000 persons aged 60–70 years [33]. According to another estimate, annual LDCT screening would induce approximately 6.7 lung cancers per year in 100,000 persons if male current smokers aged 60 years undergo annual screening until age 75 years with a compliance rate of 50% [34]. In contrast, because our population with a median age of 64 years undergoes LDCT screening twice a year, the risk of radiation-induced malignancy would be slightly higher. However, assuming that our semiannual screening yielded 57 lung cancers in 1877 participants during a median follow-up period of 3.5 years, the yearly incidence of lung cancer in 100,000 participants would be 868. Furthermore, because the 13 incidence invasive adenocarcinomas detected with the benefits of a stage shift and a size shift in our study suggest an incidence of 198 cancers per year per 100,000 persons, which is far larger than that of radiation-induced lung cancers, we maintain that semiannually repeated LDCT screening is beneficial despite the potential harm of the radiation exposure.

In conclusion, we have demonstrated that both a stage shift and a size shift occur for invasive lung adenocarcinoma during long-term repeated LDCT screening in a high-risk cohort. Long-term repeated screening for more than 5 years might disclose the potential efficacy of LDCT screening for lung cancer as the truth has been disclosed for other types of cancers, including colorectal cancer and breast cancer.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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顕微鏡検査のコツ——臨床に役立つ形態学

Ⅴ章　細胞診

総論

3　染色法

3　免疫組織化学(酵素抗体法)

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検　査　と　技　術

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総論

3 染色法

3——免疫組織化学(酵素抗体法)

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はじめに

1966年，NakaneとPierceによって，組織標本に対する酵素標識抗体法(免疫染色)が報告されてから，現在の病理組織診断にとって欠かすことのできない手法となっている．この手法は1980年頃より細胞診領域にも応用されるようになり，近年では組織診と同様に腫瘍の診断，組織型推定，原発巣の推定，悪性度評価，病原体検索などの目的で用いられる重要な手法となっている．特に数多く標本作製が可能な体腔液細胞診では，パパニコロウ(Papanicolaou)染色，ギムザ(Giemsa)染色，粘液染色などの特殊染色を用いた形態学的所見のみではなく，免疫染色を用いることにより組織型・原発巣がある程度わかり，臨床へのさらなる情報提供を可能とした．

そこで，本稿では細胞診材料における免疫染色の中でも体腔液細胞診材料に対する免疫染色を中心に述べたい．

1 免疫組織化学染色と免疫細胞化学染色の違い

細胞診材料を用いた免疫細胞化学染色はホルマリン固定，パラフィン包埋の組織切片を用いた免疫組織化学染色法と異なり，アルコール湿固定標本に対して行うため，脱パラフィン操作は不要となる．しかし，細胞診材料は湿固定標本であることから，立体構造を保持したまま固定されているため，抗原局在部位に試薬が浸透しにくい場合もあり，必ずしも安定した結果が望めないことを認識しておく必要がある．

また，脱水固定であるアルコール固定ではホルマリンなどのアルデヒド基を有する固定液で生じるような強固な架橋結合はもたらさないが，蛋白周囲の保水が取り除かれた後に，分子間結合していた官能基が分子相互間の親和性によりイオン結合，疎水結合，水素結合を形成することから，実際にはマスキングが生じうると考えられている[1]．そのことから，エストロゲンレセプター，プロゲステロンレセプター，p53蛋白などの核内抗原は賦活化操作なしで陽性像を得ることが困難な場合もあり，その回避法として組織標本同様に加熱処理を利用した抗原賦活化が有効である．

組織検体のホルマリン長期固定は抗原性失活の原因となることが知られているが，細胞診材料も同様に長期間のアルコール固定保存で抗原性が減弱するため，抗原性保持の取り扱いには細胞診材料に対しても慎重にならなくてはならない．

2 限られた細胞診材料の有効利用

細胞診の免疫染色は基本的にパパニコロウ染色の形態観察後行うが，細胞診材料では標本枚数や標的細胞数に限りがある．数に限りのある標的細胞を有効に利用する方法として，1度染色されたスライドガラスから細胞を剝離し，別のスライドに貼り付ける細胞転写法が有用である．この手法を用いれば，腫瘍細胞が標本中に出現しているスライドが1枚しかない場合でも，細胞量が多けれ

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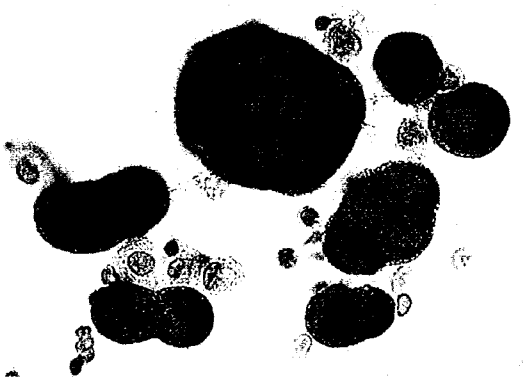

図1　胸水中の悪性中皮腫細胞(抗 calretinin 抗体)
悪性中皮腫細胞は核，細胞質に強陽性を示し，周りの組織球は陰性である．

ば複数のスライドに貼り分けることによって，複数の抗体による免疫染色が可能となる．

3 免疫細胞化学染色の細胞診への応用

1．悪性中皮腫

悪性中皮腫とは，アスベスト(石綿)曝露との関連疾患として疫学的に証明され，世界的にも重要な社会問題となっている疾患である．日本においても，高度経済成長期頃からアスベスト繊維が大量に利用されていたため，悪性中皮腫の発生が2035～2040年にピークを迎えると推測されている．

体腔液中に出現する悪性中皮腫細胞の診断は難しく，パパニコロウ染色やギムザ染色を用いた形態観察のみでは組織球，中皮細胞，腺癌細胞との鑑別が困難な場合もあり，多彩な補助的手法を併用していかなければ確定診断ができない．その中でも悪性中皮腫の診断には免疫染色によるマーカー検索が必須であり，中皮細胞マーカーとしてのcalretinin(図1)，mesothelin，cytokeratin 5/6，HBME-1，thrombomodulin，D2-40(podoplanin)，中皮細胞陰性マーカー(腺癌マーカー)のcarcinoembryonic antigen(CEA)，thyroid transcription factor-1(TTF-1)，Leu-M1，MOC-31を用いた抗体パネルの結果を考慮しながら診断することが求められている[2]．

2．腺癌マーカー(原発巣推定)

体腔液中に出現する癌細胞の形態観察から原発巣を推定することは難しいが，組織診断時の原発巣検索に用いられる免疫染色を応用することにより，ある程度まで絞り込むことが可能となる．組織診ではcytokeratin 7(CK7)とcytokeratin 20(CK20)を用いた鑑別が報告されており，CK7＋/CK20＋は膵癌，移行上皮癌，卵巣粘液性腺癌，CK7＋/CK20－は肺腺癌，悪性中皮腫，乳癌(乳管癌および小葉癌)，唾液腺癌，甲状腺癌，子宮内膜癌，卵巣漿液性腺癌，CK7－/CK20＋は大腸癌，Merkel細胞癌，CK7－/CK20－は肝細胞癌，腎癌，前立腺癌，肺小細胞癌，食道の扁平上皮癌と鑑別が可能と報告[3]され，この手法は細胞診材料にも十分応用が可能である．また，そのほかにも乳癌のmammaglobin(図2a)，甲状腺のthyrogloblinなど臓器特異性マーカーも有用である．

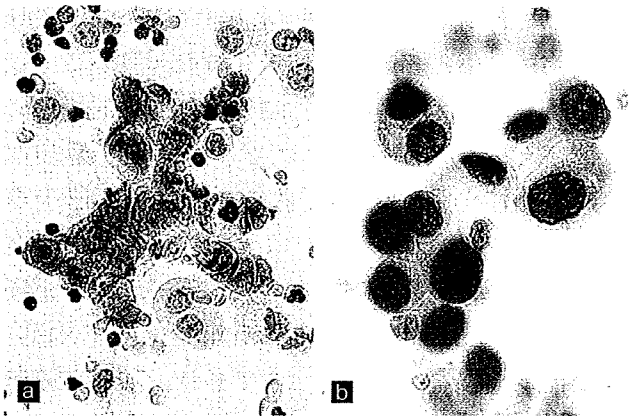

図2　胸水中にみられた腫瘍細胞
a：胸水中の乳癌細胞抗mammaglobin抗体．腫瘍細胞の細胞質に陽性像を示す．b：胸水中の肺腺癌細胞抗TTF-1抗体．腺癌細胞の核に陽性像を示す．

しかし，これらのマーカーはcytokeratinと同様，細胞質に陽性像をとるため，立体的集塊で出現することの多い細胞診材料では陽性・陰性の判定に苦慮することも少なくない．そのことから，細胞診材料では細胞質に陽性像を呈するマーカーよりも細胞核内に陽性像をみることができるマーカーのほうが判定しやすく，肺腺癌におけるTTF-1，卵巣漿液性腺癌のp53，Wilm's tumor-1(WT-1)，卵巣明細胞癌に特異性の高いhepatocyte nuclear factor-1β(HNF-1β)，細胞質および細胞膜に陽性像を呈するglypican-3，大腸癌に対するcaudal-type homeobox-2(CDX-2)などが有用である．特にTTF-1は胸水中に出現する反応性中皮細胞，肺腺癌，悪性中皮腫の識別

に極めて有用性が高い(図2b). TTF-1は甲状腺特異的に発現する遺伝子の転写調節因子として見いだされたが，肺特異分化誘導遺伝子の活性化にも関与し，正常組織においては，肺のII型肺胞上皮細胞，クララ(Clara)細胞，甲状腺濾胞上皮細胞に特異的発現を示す．このTTF-1蛋白の発現は肺腺癌および小細胞癌，大細胞神経内分泌癌(large cell neuroendocrine carcinoma，LCNEC)などにみられることが知られており，肺原発腫瘍の推定に有用なマーカーである．

おわりに

現在，保険収載されていない細胞診領域の免疫染色であるが，今後，爆発的な患者数増加が予想される悪性中皮腫の診断時には，必須の検索方法となっていることからも，細胞診断時の免疫染色の応用が増えるであろう．また，これからの細胞診断学は腫瘍の存在診断，組織型推定のみならず，治療方針決定や予後推定にも貢献することが求められる．個々の患者の診療に有用な最大限の情報を提供するためにも免疫染色は重要であり，検索精度の検証とともに応用範囲のさらなる発展が期待される．

一方，細胞診断はあくまでパパニコロウ染色による形態観察が基本であり，免疫染色の結果をもとにした形態へのフィードバックによって，さらなる細胞診断能力の向上が期待できるものと考える．

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Comparison of Different Clones (WT49 Versus 6F-H2) of WT-1 Antibodies for Immunohistochemical Diagnosis of Malignant Pleural Mesothelioma

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Abstract: Malignant pleural mesothelioma (MPM) is known to mimic the morphology of a number of diverse neoplastic conditions. WT-1 protein is conventionally used as a positive mesothelioma marker. Recently, a new monoclonal antibody clone WT49 has recently become commercially available. To compare specificity and sensitivity of the conventionally used clone 6F-H2 for the diagnosis of MPM to those of the new clone WT49. Forty cases of MPM, and 55 cases of lung carcinoma, 10 cases of synovial sarcoma of the intrathoracic region were analyzed. Of the 40 cases of MPM tested, clone WT49 and 6F-H2 stained 30 (75.0%) and 26 (65.0%) cases, respectively. Nuclear staining of clone WT49 was observed in 4 (7.2%) cases of lung carcinomas and in 1 (10.0%) case of synovial sarcoma. However, there was no nuclear staining of clone 6F-H2 in lesions other than MPM. There was no cytoplasmic staining of clone WT49 in any tumor. However, cytoplasmic staining of clone 6F-H2 was observed in 7 (17.5%) cases of MPM, 17 (30.1%) cases of lung carcinomas, and 5 (50.0%) cases of synovial sarcoma. The main advantage of WT49 is its higher reactivity with the sarcomatoid area of biphasic mesothelioma, but the results also indicate 1 drawback, that this clone was seen to react with a small percentage of lung carcinomas when it is used to distinguish epithelioid mesotheliomas from lung carcinomas. Furthermore, the positive reaction of clone WT49 was restricted to nucleus without cytoplasmic staining, which is seen in conventionally used WT-1 antibodies.

Key Words: WT-1, malignant pleural mesothelioma, lung carcinoma, immunohistochemistry

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Malignant pleural mesothelioma (MPM) is a malignant tumor characterized by a unique growth pattern in which the serosa becomes a diffusely thickened pleural "peel" encasing the lung. MPM is known to mimic the morphology of a number of diverse reactive and neoplastic conditions. In this situation, a battery of immunohistochemical markers has been used for the differential diagnosis of MPM from other chest or pleural malignant tumors. Some of the markers most widely used for MPM are polyclonal calretinin, Wilms tumor suppressor gene (WT-1),[1] cytokeratin 5/6,[2] and D2-40.[3] However, previous reports have indicated that the sensitivities and specificities of these markers vary, and no single marker has been demonstrated to be absolutely discriminatory.

WT-1 is normally expressed in only a small number of developing human organs, including the kidneys, gonads, spleen, ovarian surface epithelium, and mesothelium,[4] and is thought to play an essential role in urogenital development.[5] The precise function of WT-1 in embryonic development outside the urogenital system is, however, unclear. A number of WT-1 target genes have been identified, many of which may be relevant for tumorigenesis.[6,7]

WT-1 protein is widely used as positive mesothelioma marker. Depending on the type of antibody used, the nuclear positivity for this marker is reported to range from 43% to 98.8% in epithelioid mesotheliomas.[1,3,8–17]

Recently, a new monoclonal antibody clone named WT49 has become commercially available. This clone recognizes a prokaryotic recombinant protein containing 1 to 181 amino acids of the N-terminal of the Wilms tumor protein. However, this clone has not yet been fully evaluated by immunohistochemistry. The present study compared the specificity and sensitivity of clone 6F-H2, which is conventionally used for the diagnosis of MPM, with those of the new clone WT49.

MATERIALS AND METHODS

Case Selection

The specimens used in the present study were obtained from cases deposited in the pathology files of

the National Cancer Center Hospital, Tokyo. These samples were obtained from 40 cases of MPM (epithelioid: 28 cases, biphasic: 12 cases), and 55 cases of lung carcinoma (squamous cell carcinoma: 20 cases, adenocarcinoma: 20 cases, pleomorphic carcinoma: 15 cases), 10 cases of synovial sarcoma of the intrathoracic area (primary: 3 cases, metastasis to the lung: 7 cases). All of the diagnoses were based on the conventional histopathologic features evident in slide preparations stained with hematoxylin and eosin, some special stains, immunohistochemical, and molecular techniques available at that time.[18,19]

Immunohistochemistry

For immunohistochemical staining, 4-μm thick sections were deparaffinized and treated with 3% hydrogen peroxide for 30 minutes to block endogenous peroxidase activity, followed by washing in deionized water for 2 to 3 minutes. Heat-induced epitope retrieval with Target Retrieval Solution High pH (DAKO, Carpinteria, CA) was performed. After the slides were allowed to cool at room temperature for 40 minutes, they were rinsed with deionized water and then washed in phosphate-buffered saline for 5 minutes. The slides were then incubated with primary antibodies against WT49 (1:40, Novocastra, Newcastle upon Tyne, UK) and clone 6F-H2 (1:100, Boehring-Mannheim, Indianapolis, IN) for 1 hour at room temperature. Immunoreactions were detected using the Envison-plus system (DAKO), and visualized with 3, 3′-diaminobenzidine, followed by counterstaining with hematoxylin. Appropriate positive and negative controls were used for each antibody.

Immunohistochemical staining was scored independently by 2 observers (K.T. and N.T.). Staining was evaluated to include both the nuclear and cytoplasmic areas on a sliding scale of 0 to 3+ to represent the percentage of positive tumor cells (0 = < 1%, 1+ = 1% to 25%, 2+ = 26% to 50%, 3+ = > 51%) and the staining intensity was graded on a scale of 0 to 3 (0, negative; 1, weak; 2, moderate; and 3, strong). Positive staining was defined as score 1, 2, and 3 in both intensity and area. WT-1 immunoreactivity was further evaluated according to the histologic subtype (epithelioid or sarcomatoid area) in biphasic mesothelioma. Disagreements in judgment were resolved by means of a joint review of the slides using a multiheaded microscope.

The positivity rate between WT49 and 6F-H2 was compared using the Mann-Whitney *U* test (SPSS version 12.0, SPSS Inc, Chicago).

RESULTS

Of the 40 cases of MPM, clone WT49 and 6F-H2 stained 30 (75.0%) cases and 26 (65.0%) cases, respectively; clone WT49 showed a slightly higher frequency of immunostaining ($P = 0.332$; Table 1 and Fig. 1) than 6F-H2 (Table 2 and Fig. 2). However, a diffuse staining pattern, defined as > 50% of tumor cells stained and, an absence of differences in the positivity rate between clone WT49 (53.3%) and 6F-H2 (53.8%) were observed for all nuclear positive cases of MPM. The relationship between intensity and staining area is shown in Figures 3A and B. Clone WT49 showed strong intensity in 70% of the immunopositive cases, and clone 6F-H2 showed strong intensity in 65.4% of all immunopositive MPM cases.

TABLE 1. Nuclear Immunoreactivity of Clone WT49

	n	Positive (%)	Staining Area 0	1+	2+	3+
Mesothelioma, all subtypes	40	75.0	10	8	6	16
Epithelioid	28	78.6	6	3	4	15
Biphasic	12	66.6*, 41.6†	4*, 7†	5*, 3†	2*, 1†	1*, 1†
Lung carcinoma	55	7.2	51	3	0	1
Squamous cell carcinoma	20	10.0	18	2	0	0
Adenocarcinoma	20	0	20	0	0	0
Pleomorphic carcinoma	15	13.3	13	1	0	1
Synovial sarcoma	10	10.0	9	0	0	1

*Epithelioid areas.
†Sarcomatoid areas.

WT-1 immunoreactivity was further evaluated according to histologic subtype. Clone WT49 and 6F-H2 stained 22 (78.6%) and 20 (71.4%) cases of epithelioid mesothelioma ($P = 0.579$), and 8 (66.6%) and 6 (50.0%) epithelioid areas of biphasic mesothelioma ($P = 0.331$), respectively. Furthermore, clone WT49 showed higher positivity (41.6%) than 6F-H2 (8.3%) in the sarcomatoid area of biphasic mesothelioma ($P = 0.052$).

Discrepancies between the immunohistochemical results obtained with clone WT49 versus 6F-H2 were observed in 6 (15%) of 40 cases of MPM. Among the 40 cases examined, 5 cases (12.5%) were WT49 (+)/6F-H2 (−) and only 1 case (2.5%) was WT49 (−)/6F-H2 (+).

Nuclear staining of clone WT49 was observed in 4 (7.2%) cases of lung carcinomas (Fig. 4) and in 1 (10.0%)

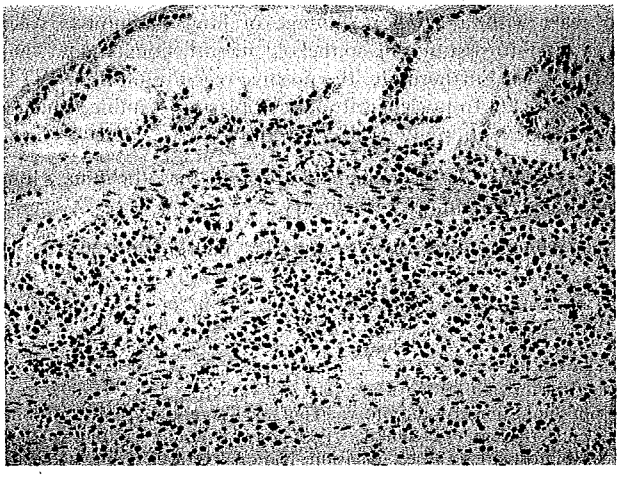

FIGURE 1. Malignant pleural mesothelioma, demonstrating papillo-tubular growth. Clone WT49 showed diffuse and strong nuclear staining for mesothelioma (original magnification × 10).

TABLE 2. Nuclear Immunoreactivity of Clone 6F-H2

	n	Positive (%)	Staining Area 0	1+	2+	3+
Mesothelioma, all subtypes	40	65.0	14	5	7	14
Epithelioid	28	71.4	8	2	5	13
Biphasic	12	50.0*, 8.3†	6*, 11†	3*, 0†	2*, 1†	1*, 0†
Lung carcinoma	55	0	55	0	0	0
Squamous cell carcinoma	20	0	20	0	0	0
Adenocarcinoma	20	0	20	0	0	0
Pleomorphic carcinoma	15	0	15	0	0	0
Synovial sarcoma	10	0	10	0	0	0

*Epithelioid areas.
†Sarcomatoid areas.

case of synovial sarcoma. However, in clone 6F-H2 no nuclear staining of lesions was observed other than for MPM. The overall specificity of clone WT49 and 6F-H2 for MPM was 92.3% and 100%, respectively.

There was no cytoplasmic staining of clone WT49 in any tumor. However, cytoplasmic staining of clone 6F-H2 was observed in 7 (17.5%) cases of MPM, 17 (30.1%) cases of lung carcinoma in (Table 3, Fig. 5), and 5 (50.0%) cases of synovial sarcoma. Furthermore, cytoplasmic staining with clone 6F-H2 was observed in blood and lymphatic vessels in all specimens and some striated muscle in which the lesion was resected along with portions of the chest wall.

DISCUSSION

The current study demonstrated 2 advantages and 1 disadvantage of clone WT49 in comparison to clone 6F-H2. The first advantage is that clone WT49 showed higher sensitivity (75.0%) than clone 6F-H2 (65.0%), although there was no statistical difference. Specifically, in the sarcomatoid area of biphasic mesothelioma, clone WT49 showed a statistically marginally higher positivity rate (41.6%) than that of 6F-H2 (8.3%; $P = 0.052$). The sensitivity of WT-1 for MPM has been reported to range from 43% to 96%.[1,3,8–14] These reports used rabbit polyclonal antibody against WT-1 and/or monoclonal antibody clone 6F-H2. The utility of clone WT49 immunostaining has not yet been investigated fully. These findings demonstrated that clone WT49 has a sensitivity that is as good as or better than that of clone 6F-H2.

The relationship between the intensity and staining area showed that as the staining area became wider, the staining intensity became stronger with both clones. However, 2 cases of +1 staining area showed strong intensity in clone WT49 but not in clone 6F-H2. These findings indicate that clone WT49 has a tendency toward slightly stronger immunoreactivity in comparison to that of 6F-H2 when only a tiny area is positive. Clone WT49

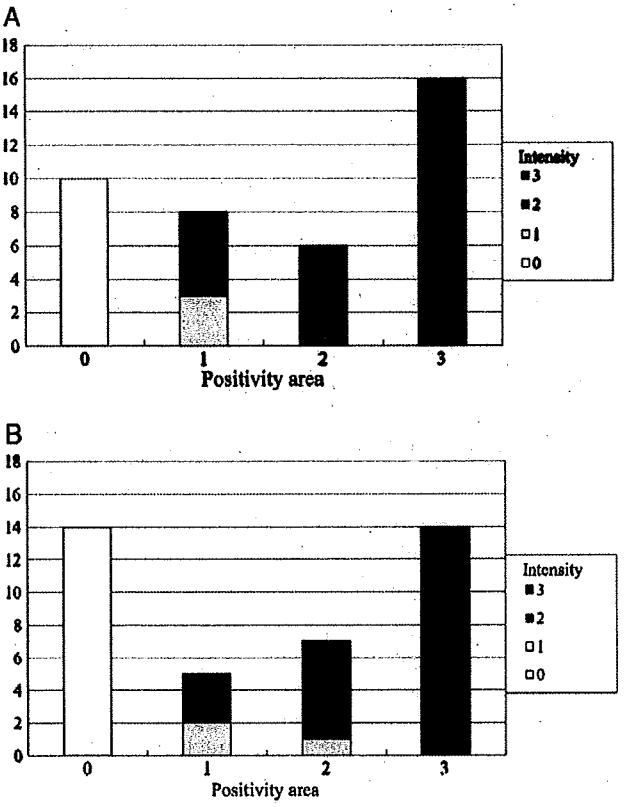

FIGURE 3. A, Correlation of immunoreactive area and staining intensity for clone WT49. The white bar indicates negative (0), the light gray bar indicates mild intensity (+1), the dark gray bar indicates moderate intensity (+2), and the black bar indicates strong intensity (+3). B, Correlation of immunoreactive area and staining intensity for clone 6F-H2. The white bar indicates negative (0); light gray bar, mild intensity (+1); dark gray bar, moderate intensity (+2); black bar, strong intensity (+3).

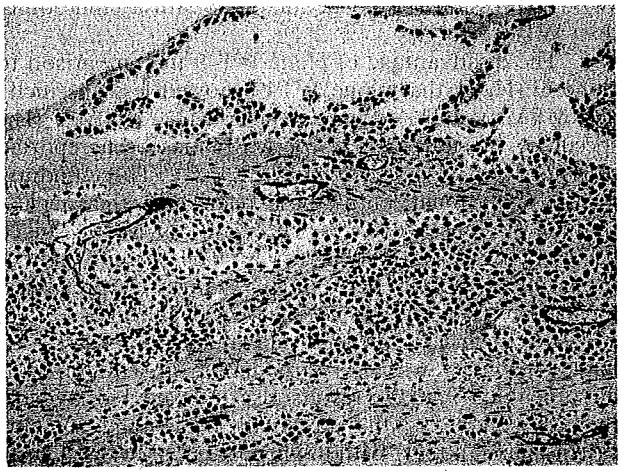

FIGURE 2. Same case as shown in Figure 1. Clone 6F-H2 showed not only diffuse and strong nuclear staining for nucleus of mesothelioma but also evident staining of blood and lymphatic vessels (original magnification ×10).

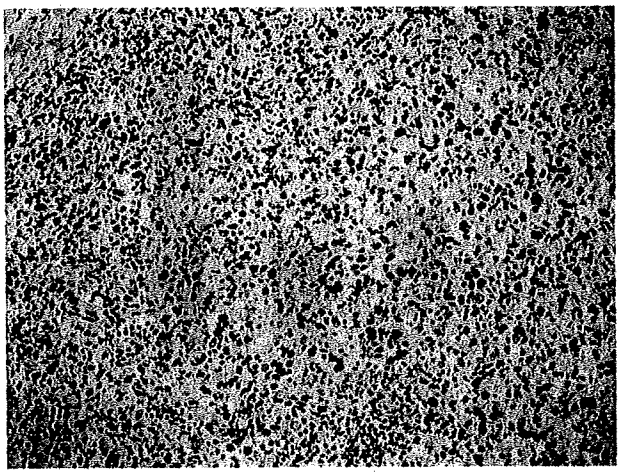

FIGURE 4. Clone WT49 showed diffuse and strong nuclear staining in the nucleus of pleomorphic carcinoma of the lung (original magnification × 10).

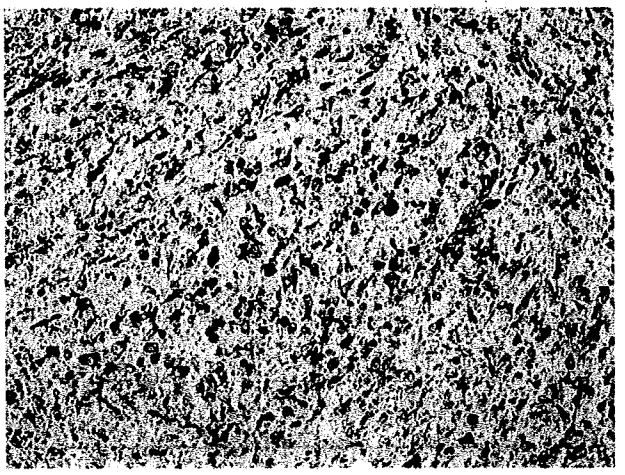

FIGURE 5. Clone 6F-H2 showed diffuse and strong cytoplasmic staining of pleomorphic carcinoma of the lung (original magnification × 10).

may have an advantage when the diagnosis is based on a limited sample, such as a biopsy specimen.

The second advantage of clone WT49 is the ease of judgment for immunostaining. The positive reaction of clone WT49 was restricted to the nucleus. Cytoplasmic staining by the other WT-1 antibodies has been reported. WT1 is principally a DNA-binding transcription factor mainly distributed in the nucleus; therefore, cytoplasmic staining has been regarded as nonspecific and has not been counted as positive in most previous reports.

One of the causes of inappropriate cytoplasmic immunohistochemical staining is the presence of endogenous biotin. To avoid the problem of nonspecific endogenous biotin staining, the EnVision+ detection system was employed in the immunohistochemical staining procedure. EnVision+ is a biotin-free detection method that uses a secondary antibody covalently linked to dextrose polymers coated with peroxidase molecules.

TABLE 3. Cytoplasmic Immunoreactivity of Clone 6F-H2

			Staining Area			
	n	Positive (%)	0	1+	2+	3+
Mesothelioma, all subtypes	40	17.5	33	3	1	3
Epithelioid	28	10.7	25	0	0	3
Biphasic	12	20.0*, 25.0†	10*, 9†	1*, 3†	1*, 0†	0*, 0†
Lung carcinoma	55	30.1	38	14	2	1
Squamous cell carcinoma	20	10.0	18	2	0	0
Adenocarcinoma	20	30.0	14	5	1	0
Pleomorphic carcinoma	15	60.0	6	7	1	1
Synovial sarcoma	10	50.0	5	1	3	1

*Epithelioid areas.
†Sarcomatoid areas.

Therefore, the cytoplasmic staining of clone 6F-H2 in the present study is not caused by endogenous biotin.

One of the reasons for the cytoplasmic distribution of WT-1 is because phospholylation in the DNA-binding domain of WT-1 alters the affinity for DNA and the subcellular distribution of WT-1.[20] Recently, an immunohistochemical study demonstrated that WT-1 is expressed in a wide variety of human malignancies, including those of the gastrointestinal and pancreatobiliary, urogenital and respiratory tracts, neuronal system and mesenchymal tissues when cytoplasmic staining counted as positive.[21] Therefore, WT-1 is now regarded as a molecular target of immunotherapy for various malignant tumors. A clinical trial of a WT-1 peptide-based cancer immunotherapy is ongoing.[22] In judging the eligibility of a patient for this immunotherapy, WT-1 expression should be analyzed with a clone other than WT49.

The disadvantage of clone WT49 in comparison to clone 6F-H2 is that clone WT49 showed less specificity for MPM (92.3%) than clone 6F-H2 (100%). The specificity of clone 6F-H2 for MPM has been reported to range from 80% to 100%.[1,3,8,11,12,15,21] These findings include polyclonal antibodies, restricting the nuclear staining of clone 6F-H2, the specificity for MPM ranged between 84.3% and 100%.[1,3,8,15,21] The current findings using clone WT49 were in the range of previously reported results for conventional WT-1 antibodies.

In conclusion, the main advantage of WT49 is its higher reactivity with the sarcomatoid area of biphasic mesothelioma, but the results also indicate 1 drawback, that this clone is that a small percentage of lung carcinomas can react with it when it is used to distinguish epithelioid mesotheliomas from lung carcinomas. Furthermore, the positive reaction of clone WT49 was restricted to the nucleus without the cytoplasmic staining observed with conventionally used WT-1 antibodies.

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我が国における中皮腫，石綿肺がんの臨床像

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水橋　啓一[4]，大西　一男[5]，玄馬　顕一[2]，藤本　伸一[2]

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[2]岡山労災病院

[3]北海道中央労災病院

[4]富山労災病院

[5]神戸労災病院

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メインシンポジウム2

我が国における中皮腫，石綿肺がんの臨床像

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（平成21年3月9日受付）

要旨：平成12年以降，平成20年1月までに全国労災病院で診断，治療した中皮腫221例，石綿肺がん135例の臨床像を検討した．方法は，労災補償制度に精通した呼吸器専門医が各労災病院を訪問して症例を収集した．中皮腫221例のうち胸膜中皮腫は83.3%，腹膜中皮腫は13.1%であった．胸膜中皮腫症例で石綿ばく露を疑う職歴は85.4%にみられ，その職種は造船所内の作業，建設作業，配管作業，断熱作業などであった．平均年齢は66.9歳で男性が85.9%であった．組織型は上皮型52%，肉腫型28%，二相形14%であり，生存期間中央値は全症例では7.3カ月，手術例では11.1カ月であった．石綿ばく露に関連する画像所見は胸水が82.2%にみられ，胸水中のヒアルロン酸値が100,000ng/ml以上であったのは39.4%であった．石綿肺がん症例の平均年齢は71.1歳で，男性が97.0%であった．組織型は腺がん57.5%，扁平上皮がん29.1%，小細胞がん12.6%であり，生存期間中央値は9.7カ月，5年生存率は18.0%で一般の肺がんと同様の傾向であった．石綿肺がん症例の方が，中皮腫症例に比べ，石綿肺所見，胸膜プラークの比率が高く，また，石綿ばく露期間が長いことより，石綿肺がん症例は中皮腫症例に比べ職業性石綿ばく露が高濃度であることが示唆された．両疾患とも予後が悪く，現状では早期発見による手術症例を増やすことが重要である．

（日職災医誌，57：190—195，2009）

—キーワード—
中皮腫，石綿肺がん，臨床像

はじめに

平成16年度から労働者健康福祉機構の労災病院の我々のグループは石綿粉じんを含む粉じんばく露による肺がんの研究を開始した．平成16年6月，クボタ旧神崎工場周辺の住民に中皮腫が多発し，見舞金が支払われたとの報道がなされて以来，石綿による疾病が社会問題化し，平成18年4月には石綿関連の研究を独立させ体制の強化をはかった．その研究の一環で「石綿ばく露による肺がんおよび悪性中皮腫例の調査研究」を行い，我が国における中皮腫，石綿肺がんの臨床像を明らかにしたので報告する．

対象と方法

中皮腫は，平成12年以降，平成20年1月までに全国27労災病院で臨床または病理学的に中皮腫と診断された症例を対象とした．石綿肺がんは同時期に石綿肺がんとして労災補償された症例，または石綿健康被害救済法で救済された症例を対象とした．方法は，労災補償制度に精通した呼吸器専門医が全国労災病院を訪問し，カルテ，画像，病理標本などを調査し，その資料を岡山労災病院に送付して検討した．調査項目は，年齢，性別，転帰，発見契機，職歴，発生部位，組織型，画像所見，石綿小体数，胸水中ヒアルロン酸値，病期分類，診断方法，治療方法などであり，中皮腫の病期分類は1995年のInternational Mesothelioma Interest Group（IMIG）分類[1]を

表1　中皮腫症例の背景因子

部位	胸膜	腹膜	心膜	精巣鞘膜	計
症例数 (%)	184 (83.3%)	29 (13.1%)	4 (1.8%)	2 (0.9%)	219*
年齢：					
平均値	66.9	63.0	58.8	51.5	66.1
標準偏差	10.0	12.0			10.5
中央値	67	67			67
最小値	38	30			30
最大値	92	80			92
性別：					
男性	158	22	1	2	183
女性	26	7	3	0	36

*：部位不明2例を除く

表2　胸膜中皮腫症例の発見契機および自覚症状

発見契機	症例数 (%)
症状発見	135 (73.4%)
胸痛・背部痛	55
呼吸困難	53
咳嗽	24
発熱	12
その他	9
健診発見	27 (14.7%)
他疾患治療中など	22 (12.0%)

症状には重複あり

表3　胸膜中皮腫の診断方法

診断方法	症例数
組織診	168
開胸術	28
VATS	58
局所麻酔下胸腔鏡	35
針生検	31
Cope針	7
剖検	4
不明	5
細胞診	11
不明	5

表4　胸膜中皮腫のStage分類

	Stage I	Stage II	Stage III	Stage IV	計
症例数	40	13	64	62	179
治療法：					
切除術	18	6	19	1	44
化学療法	3	3	30	37	73
対症療法	15	4	13	24	56
不明	4	0	2	0	6

Stage分類不明の5例を除く

用いた．

職業性石綿ばく露については，作業内容，初回ばく露年齢，ばく露期間，および，初回ばく露から中皮腫または肺がん発生までの潜伏期間を検討した．石綿ばく露の客観的指標として，胸部画像における石綿肺，胸膜プラーク，びまん性胸膜肥厚，円形無気肺の所見の有無につき検討した．所見は呼吸器内科医2名と放射線科医1名の合計3名の合議制で決定した．さらに，手術あるいは剖検が可能であり肺組織が得られた症例については，神山変法[2]を用いて肺内石綿小体数を測定した．

結　果

中皮腫の臨床像

収集された症例数は221例であった．胸膜中皮腫は184例で，男性158例，女性26例，年齢中央値は67歳であった(表1)．発見契機は症状発見135例，健診発見27例，他疾患治療中22例であり，自覚症状は胸痛，呼吸困難などであった(表2)．組織診が168例になされ，その方法は開胸術，VATS，局所麻酔下胸腔鏡，針生検などであった(表3)．組織型は上皮型91例，肉腫型49例，二相形25例であり，10例は分類不能であった．胸水中ヒアルロン酸値は100,000ng/mlをカットオフ値とした場合，94例中37例(39.4%)が陽性であった．病期分類は，Stage I 40例，Stage II 13例，Stage III 64例，Stage IV 62例であり，治療法は，切除術44例，化学療法73例，対症療法56例，不明6例であった(表4)．生存期間中央値は7.3カ月であり(図1)，手術例と非手術例の比較ではそれぞれ11.1カ月，8.0カ月で両者の生存率に有意差を認めた($p<0.05$)(図2)．

石綿ばく露が疑われる職歴は85.4%に聴取され，その内訳は造船所内の作業，建設作業，配管作業，断熱作業などであった(表5)．初回ばく露年齢，期間，潜伏期間の中央値はそれぞれ21歳，30年，43年であった(表6)．画像所見は胸水貯留148例，胸膜プラーク92例などであった(表7)．石綿小体は45例で測定され，乾燥肺1gあたりの本数別の内訳は1,000本未満10例，1,000本～4,999本10例，5,000本以上25例であった(表8)．

石綿肺がんの臨床像

対象症例は135例であった．男性131例，女性4例で，平均年齢71.1歳±9.0歳，中央値72歳であった．発見契機は症状発見59例，健診発見50例，他疾患治療中18例であり，症状は呼吸困難19例，咳12例などであった．喫煙指数は，非喫煙者12例，喫煙者113例であり，そのうち600以上の重喫煙者が93例であった．組織型は，腺がん73例，扁平上皮がん37例，小細胞がん16例，大細胞がん1例であり，治療内容は切除術50例，化学療法44例，放射線療法7例，化学療法および放射線療法14例，対症療法17例であった．生存期間の中央値は9.7カ月であり(図3)，治療法別の中央値は，外科手術17.5カ月，化学療法7.7カ月，放射線療法8.0カ月，化学療法および放射線療法9.6カ月，対症療法3.2カ月であった(図4)．職業歴の内訳は中皮腫と同様であり，初回ばく露年齢，