of the PET scanner in 12 patients (maximum tumor focus dimensions in false-negative LNs ranging from 1 to 7.5 mm, with an average of 3.4 mm); (2) PET mis-localization of a mediastinal LN as a hilar LN in one patient; (3) weak FDG uptake by microscopic tumor foci due to necrosis with massive bleeding in a metastatic LN in one patient. Conclusions: Inflammatory conditions were most responsible for false-positive PET scans, and spatial resolution limitation of FDG PET was the causative factor of false-negative PET scans. Recognizing these factors in advance would be clinically helpful in accurate nodal staging with FDG PET.

1. Introduction

Accurate lymph node (LN) staging is critical in determining treatment strategy and predicting outcome for patients with non-small cell lung cancer (NSCLC). Patients without metastatic LN (N0) or with only intrapulmonary or hilar LN involvement (N1) are candidates for straightforward surgical resection. Patients with ipsilateral metastatic mediastinal LN (N2) are usually treated with chemoradiotherapy or with induction therapy followed by surgery. Patients with further node involvement (N3) are not considered for primary surgery, and chemo-radiotherapy is indicated.

Computed tomographic (CT) scanning is the most widely accepted non-invasive imaging modality for nodal staging in NSCLC patients, but it is far from satisfying and is less accurate than invasive surgical staging such as mediastinoscopy, mediastinotomy, or video-assisted thoracoscopic surgery [1,2]. CT is an anatomic imaging tool that demonstrates LN enlargement as a sign of metastasis. It is generally agreed that normal-sized LNs can harbour metastases while enlarged LNs can be metastasis-free [3–6]. It is not possible to accurately diagnose LN status based on CT measurement alone.

By contrast, positron emission tomography (PET) with fluorine-18 fluorodeoxyglucose (FDG) provides functional images of glucose uptake and phosphorylation [7]. Meta-analytic studies [8–11] have demonstrated that PET was significantly more accurate than CT for nodal staging in NSCLC. Although FDG PET represents an important advance in NSCLC nodal staging, there are some false-positive and false-negative results in any studies. Thus, invasive surgical staging still remains the gold standard for accurate nodal staging.

To date, few studies have attempted to determine the causative factors of false PET results in nodal staging in NSCLC [12–14]. Recognizing these factors in advance would be clinically helpful in accurate nodal staging with FDG PET. The aim of this study is to clarify clinico-pathologic factors responsible for false PET results.

2. Materials and methods

2.1. Patients

From July 2000 through December 2001, FDG PET was performed in 226 patients with thoracic abnormalities at the National Cancer Center Hospital East. Among them, we retrospectively reviewed 71 patients with proven or suspected NSCLC, who later had pathologic confirmation of their resected tumor and nodal status, in this study. These patients underwent PET to evaluate their indeterminate lung lesions or to determine the preoperative staging. Patients with obvious bulky mediastinal lymph adenopathy were excluded. Not all surgical candidates during the same period were included, because of limited PET examination capacity at our institute. Informed consent for FDG PET was obtained from all patients.

There were 41 men and 30 women, and their ages ranged from 36 to 90 years (median 65 years). Seventy of the 71 patients underwent major lung resection and systematic lymph node dissection. The remaining one patient underwent preoperative mediastinoscopy, which revealed mediastinal node involvement, and received combined chemoradiotherapy. No patient in this study had received induction therapy. CT, PET and surgery were carried out within 1 month in all patients.

2.2. FDG PET

All patients were asked to keep fasting for at least 6 h before FDG injection in order to minimize the blood insulin level and normal tissue glucose uptake. Serum glucose levels before FDG injection were 150 mg/dl or lower in all patients. Whole body FDG PET imaging was performed using a GE Advance Scanner (General Electric Medical System, Milwaukee, WI), which has an axial field of view of 15 cm and a spatial resolution of 4.5 mm full-width-half-maximum. PET imaging included the entire field of view from the skull base to the thigh. Sixty min-

utes after intravenous injection of 300 MBq of F-18 FDG, emission scanning was performed in 5 min, and transmission scanning in 1 min. Data acquisition was performed in seven bed positions.

All PET images were interpreted by one or two experienced nuclear medicine radiologists. The 4.25 mm-thick images were reviewed in axial, coronal, and sagittal planes on hard copy films. Based on a visual comparison between CT and PET, LN stations of abnormal uptake were determined according to Naruke's lymph node mapping [15]. LNs were considered positive for malignancy when uptake was greater than the mediastinal blood pool activity by visual estimation.

2.3. Thoracic CT

CT was performed before PET imaging in all patients. CT was done with an X-Vigor or an Aquilion (Toshiba Medical Systems, Tokyo, Japan), and contiguous 5—7.5 mm thick sections were obtained from the pulmonary apices to the bases in a supine position at full inspiration. Dynamic incremental scanning was always performed, after bolus injection of 100 ml of contrast material using an automatic injector.

All contrast-enhanced CT films were interpreted by at least two experienced thoracic radiologists. The following findings were recorded: the maximum dimension and location of the primary lesion, LN sizes, obstructive pneumonia or atelectasis owing to primary tumor, and pulmonary fibrosis. The location of a primary tumor was defined to be central when it was located in the inner one third of the lung field, and peripheral when in the outer two thirds on CT scan. LNs larger than 1 cm in the shortest dimension were considered positive for malignancy. LN stations on CT were described according to Naruke's lymph node mapping [15].

2.4. Clinico-pathologic correlation

All 71 patients had final histologic confirmation of NSCLC by reviewing tissue specimens obtained by resection (n = 70) or mediastinoscopy (n = 1). Histologic typing was determined according to the World Health Organization classification [16]. The disease stage was based on the TNM classification of the International Union Against Cancer [17].

All resected lymph nodes were formalin-fixed and examined microscopically by hematoxylin and eosin staining. The LN stations on thoracotomy were described according to Naruke's lymph node mapping [15]. To determine histologic causative factors of false PET scans, two authors (K.T. and

G.I.) compared PET results and histologic features of LNs by nodal station, and measured the maximum dimension of tumor foci in histologically involved LNs.

Clinical records of each patient were reviewed for age, gender, and a past history of inflammatory disease.

3. Results

There were 50 adenocarcinomas, 12 squamous cell carcinomas, five large cell carcinomas, three large cell neuroendocrine carcinomas, and one adenosquamous carcinoma.

FDG-uptake in primary tumors was sufficient in 65 patients and lacking in six. All PET-negative primary tumors were histologically diagnosed as adenocarcinoma with predominant bronchioloalveolar carcinoma (BAC) component, and were pathologic N0 diseases correctly staged as N0 both by CT and PET.

The nodal staging results of CT and FDG PET are presented in Table 1. There were 10 (14%) false-positive PET scans and 14 (20%) false-negative PET scans. Sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) for CT were 29, 83, 65, 47, and 70% and for PET were 39, 79, 66, 47, and 73%, respectively.

The CT and FDG PET results of the discrimination between N0/N1 and N2/N3 are presented in Table 2. Sensitivity, specificity, accuracy, positive predictive value, and negative predictive value for CT were 20, 89, 75, 33, and 81% and for PET were 40, 88, 77, 46, and 84%, respectively.

The 10 cases with false-positive PET scan are summarized in Table 3. These patients had FDG uptake in LNs compatible with metastases, but his-

Table 1 Results of nodal staging as the assessment of N1, N2, and N3 lymph nodes on CT and FDG PET

	Pathologic nodal stage		
	pN0	pN1	pN2
Nodal stage on CT			
N0	39	5	11
N1	2	4	1
N2	3	2	3
N3	0	1	0
Nodal stage on PET			
N0	38	5	4
N1	3	3	5
N2	0	3	6
N3	3	1	0

Table 2 Results of the discrimination of N0/N1 from N2/N3 on CT and FDG PET

| | Pathologic nodal stage | |
	pN0/pN1	pN2/pN3
Nodal stage on CT		
N0/N1	50	12
N2/N3	6	3
Nodal stage on PET		
N0/N1	49	9
N2/N3	7	6

tology confirmed no metastatic involvement. The causative factors for false-positive PET scan were: (1) inflammatory conditions (tumor necrosis in two patients, tumor necrosis and obstructive pneumonia in two, LN sarcoid reaction due to previous pulmonary tuberculosis in one (Fig. 1), pulmonary fibrosis in one, rheumatoid arthritis in one); (2) PET mis-localization of an interlobar LN (station #11s) as a mediastinal LN (station #4) in one patient; (3) inability to distinguish the endobronchial polypoid growth of a primary tumor from a lobar LN in one patient (Fig. 2); (4) unknown in one patient. All false-positive LNs due to inflammatory conditions showed reactive lymphoid hyperplasia histologically.

The 14 cases with false-negative PET scan are summarized in Table 4. These patients were found to have LN metastases, despite having PET scans interpreted as negative. The causative factors for false-negative PET scan were: (1) limitation of the spatial resolution of the PET scanner in 12 patients (Fig. 3); (2) PET mis-localization of a mediastinal LN (station #5) as a hilar LN (station #10) in one patient; (3) weak FDG uptake by sporadic microscopic tumor foci due to necrosis with massive bleeding in a metastatic LN in one patient (Fig. 4).
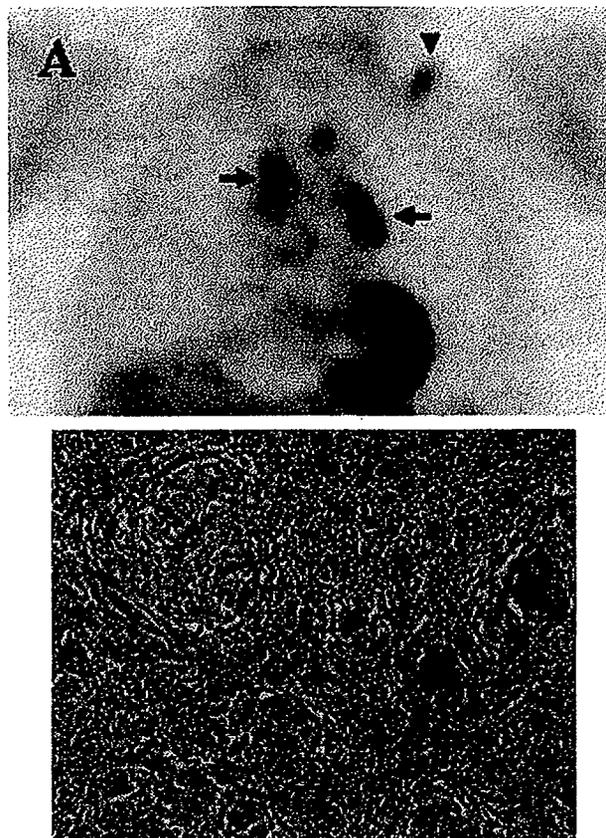

Fig. 1. A 64-year-old woman had adenocarcinoma in the left lower lobe, with a past history of pulmonary tuberculosis. CT demonstrated no enlarged lymph nodes. (A) FDG PET (coronal view) demonstrated increased FDG uptake in the bilateral hilar, mediastinal (arrow), and left supraclavicular lymph nodes (arrow head). (B) Enlarged lymph nodes harbored epithelioid granulomas with Langhans-type giant cells (arrow), which were diagnosed as sarcoid reaction (hematoxylin—eosin).

Table 3 False-positive PET scans

Patient no.	Age/sex	Histology of primary tumor	Location of primary tumor	Nodal stage by CT/PET/ pathology	Causative factors
1	43/F	P/D Ad	Peripheral	N0/N2/N1	Mis-localization of lymph nodes
2	67/F	M/D Ad	Peripheral	N2/N2/N1	Unknown
3	68/M	LCNEC	Peripheral	N0/N3/N0	Tumor necrosis
4	74/F	La	Central	N1/N1/N0	Endobronchial polypoid growth of a primary tumor
5	65/M	La	Central	N3/N2/N1	Tumor necrosis
6	73/M	La	Peripheral	N0/N1/N0	Tumor necrosis and obstructive pneumonia
7	64/F	W/D Ad	Peripheral	N0/N3/N0	Sarcoid reaction (past history of tuberculosis)
8	65/M	M/D Sq	Central	N1/N1/N0	Tumor necrosis and obstructive pneumonia
9	71/F	M/D Ad	Central	N0/N3/N0	Pulmonary fibrosis
10	59/F	M/D Ad	Peripheral	N0/N3/N1	Rheumatoid arthritis

W/D: well differentiated; M/D: moderately differentiated; P/D: poorly differentiated.

Fig. 2. A 74-year-old woman with large cell carcinoma in the left lower lobe. (A) FDG PET (coronal view) demonstrated increased FDG uptake in the primary tumor (arrow heads) and adjacent lobar lymph node (arrow, PET–N1). (B) Histologically, the involved lobar node on FDG PET corresponded to endobronchial polypoid growth of the primary tumor.

The maximum dimensions of tumor focus in false-negative LNs in group (1) ranged from 1 to 7.5 mm (mean 3.4 mm).

4. Discussion

Many retrospective [13,18] and prospective [13,19—21] studies have shown FDG PET to be an accurate imaging modality in the nodal staging of NSCLC. Meta-analytic comparisons of PET and CT [8—11] showed that PET was significantly more accurate than CT in demonstrating nodal metastases. While PET is an important advance in non-invasive staging, it is not perfect. However, few studies have assessed its limitations. In this study, we
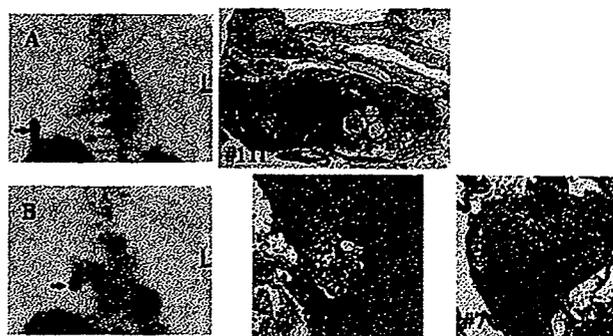

Fig. 3. A 67-year-old man with adenocarcinoma in the right lower lobe. CT demonstrated no enlarged lymph nodes. A FDG PET (coronal view) demonstrated increased FDG uptake in the (A) primary tumor (arrow) and (B) a hilar lymph node (arrow) but not in the mediastinal nodes. The maximum dimensions of tumor focus (black line) in the involved LNs were (C) 16 mm in #11i (PET-positive) (D) 1 mm in #4 (PET-negative), and (E) 4.5 mm in #7 lymph node (PET-negative).

clarified clinico-pathologic factors responsible for false PET results.

We showed that false-positive PET scans were mostly attributable to inflammatory conditions, including tumor necrosis, obstructive pneumonia, previous pulmonary tuberculosis, pulmonary fibrosis, and rheumatoid arthritis. FDG is not a specific marker of malignancy and FDG uptake can be seen at sites of active, acute inflammation, which is due to increased glucose uptake by activated macrophages and inflammatory cells [22]. Inflammatory conditions are well-known factors associated with false-positive PET scans in indeterminate pulmonary nodule evaluation [23]. Roberts et al [12] reported that concurrent inflammatory lung disease and centrally located tumors were causative factors of false-positive PET scans in mediastinal nodal staging in NSCLC. Our results were consistent with these previous findings. Because false-positive populations can benefit from primary surgery, an invasive staging procedure prior to pulmonary resection may be indicated in patients with positive-LN findings on PET, especially those with present or past inflammatory conditions.

FDG PET diagnostic capability is limited not only by cellular activity but also by tumor volume. FDG uptake by small tumor cell focus is often poorly depicted due to partial volume effect. Current PET scanner achieves transaxial resolution of 4—5 mm full-width-half-maximum. A tumor focus smaller than 5 mm may not be detected by current scanners. The maximum dimensions of tumor focus in false-negative LNs ranged from 1 to 7.5 mm (mean 3.4 mm) in our series. The spatial resolution limitations of FDG PET were responsible for 13 of 14 (93%)

Table 4 False-negative PET scans					
Patient no.	Age/Sex	Histology of primary tumor	Location of primary tumor	Nodal stage CT/PET/ pathology	Causative factors
1	59/M	M/D Ad	Peripheral	N0/N0/N2	Small-sized tumor focus (7.5 mm)[a]
2	57/F	P/D Ad	Peripheral	N0/N1/N2	Mis-localization of lymph nodes
3	64/M	M/D Ad	Peripheral	N0/N1/N2	Small-sized tumor focus (5 mm)
4	69/F	M/D Ad	Peripheral	N2/N0/N1	Small-sized tumor focus (5 mm)
5	58/M	M/D Ad	Peripheral	N0/N0/N1	Small-sized tumor focus (2 mm)
6	43/F	M/D Ad	Peripheral	N0/N0/N2	Small-sized tumor focus (1 mm)
7	74/M	M/D Sq	Peripheral	N0/N0/N1	Small-sized tumor focus (5 mm)
8	76/F	M/D Ad	Peripheral	N1/N0/N1	Small-sized tumor focus (4.5 mm)
9	65/M	M/D Sq	Peripheral	N0/N0/N2	Small-sized tumor focus (4 mm)
10	75/M	M/D Sq	Peripheral	N0/N0/N2	Small-sized tumor focus (2.5 mm)
11	73/F	W/D Ad	Central	N0/N0/N1	Small-sized tumor focus (4 mm)
12	67/M	W/D Ad	Peripheral	N0/N1/N2	Small-sized tumor focus (4.5 mm)
13	65/M	M/D Ad	Central	N2/N1/N2	Weak FDG uptake by microscopic tumor foci in a metastatic LN
14	49/M	M/D Ad	Peripheral	N1/N1/N2	Small-sized tumor focus (2.5 mm)

W/D: well differentiated; M/D: moderately differentiated; P/D: poorly differentiated.
[a] The maximum dimension of a tumor focus in false-negative lymph nodes are described in parenthesis.
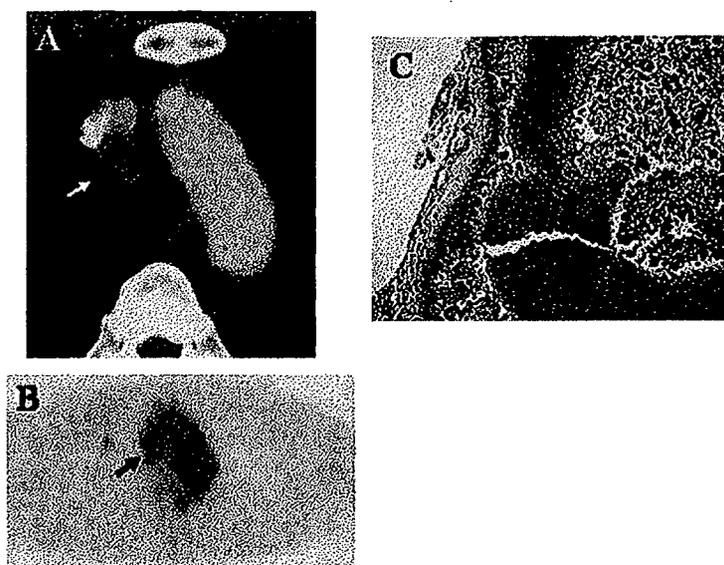

Fig. 4. A 65-year-old man with adenocarcinoma in the right upper lobe. (A) CT demonstrated an enlarged pretracheal lymph node with a low density area (arrow). (B) FDG PET (axial view) demonstrated weak FDG uptake at a pretracheal lymph node (arrow). (C) Histologically, the pretracheal lymph node showed scattered microscopic tumor foci (arrow heads) in an area of necrosis with massive bleeding.

false-negative PET results in the present study. FDG PET is not reliable in diagnosing small tumor foci in LNs. The rewarding prognosis after straightforward resection in patients with minimal N2 disease (no mediastinal LN enlargement on CT), compared to those with clinical N2 disease (mediastinal LN enlargement on CT) is well documented [24,25]. Because most false-negative LNs in our study had tiny tumor foci 5 mm or less in size and were not en-

larged on CT, fair prognosis after surgical resection can be expected. False-negative PET results might not be clinically relevant.

Another disadvantage of FDG PET is its limited anatomical resolution due to the paucity of anatomic information in metabolic images [21]. Although PET-positive LNs were localized referring to contrast-enhanced CT findings in this study, it was hard to distinguish hilar LNs from adjacent me-

diastinal LNs in two cases (one false-positive and one false-negative PET scans). In another case, we could not discriminate endobronchial polypoid tumor growth from a lobar LN. New PET—CT fusion systems may enable more precise anatomic localization [26—28].

Our estimates in the diagnostic accuracy of PET for LN staging in NSCLC patients may be biased. Our study resulted in a sensitivity of 39%, specificity of 79%, and accuracy of 66%, and did not show the superiority of PET over CT. Our sensitivity lower than many prospective studies [13,19—21] and meta-analyses [8—11] may be attributable to several factors. First, our patient population, the majority of which (55/71) had no enlarged LN on CT, might be biased. Most involved mediastinal nodes were not enlarged, which may explain too many false-negatives. Second, our method of FDG PET may not be optimal in the following points: (1) allowing patients with serum glucose values up to 150 mg/dl (2) rather low injected dose of FDG, and (3) interpretation of images on hard copy films. Third, the definition of nodal staging accuracy of PET used in our study was different from that in previous studies. We defined accurate staging as correct localization of involved N1, N2, and N3 nodes. This was because we compared PET staging results and histologic findings to determine pathologic factors responsible for false PET results. In most previous studies, however, accurate LN staging was defined as correct discrimination between N0/N1 and N2/N3. This is reasonable because mediastinal node involvement diagnosis is important in treatment strategy decision making. So, we also evaluated nodal staging accuracy as correct discrimination between N0/N1 and N2/N3. Although the specificity and NPV increased, sensitivity and PPV were almost the same. This may be attributable to the low pathologic N2 prevalence (21%) in our study population. Phillips et al [29] reported that as prevalence of disease decreases, the reliability of a positive result will drop, and the reliability of a negative result will increase. High NPV of PET in diagnosing mediastinal node involvement may warrant mediastinoscopy omission.

Although the majority of lung cancer shows increased FDG uptake, BAC has been reported to be often negative on FDG PET [30,31]. In our study, all PET-negative primary tumors were adenocarcinomas with predominant BAC component. BAC predominant adenocarcinomas rarely have lymph node metastases and distant metastases [32,33]. They are reliably diagnosed on thin-section CT scan, because they are depicted as ground glass opacity [34]. These adenocarcinomas may not be indicated for preoperative PET examinations for the characteristics.

In summary, inflammatory conditions were most responsible for false-positive PET scans, and spatial resolution limitation of FDG PET was the causative factor of false-negative PET scans. A positive PET scan cannot guarantee a pathologic positive LN, and a negative PET scan cannot guarantee a pathologic negative LN. Recognizing these factors in advance would be clinically helpful in accurate nodal staging with FDG PET.

Acknowledgments

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FROM THE ASCO-JSCO JOINT SYMPOSIUM

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Postoperative adjuvant therapy for completely resected early-stage non-small cell lung cancer

Abstract Consensus on adjuvant therapy for completely resected non-small cell lung cancer until 2002 was as follows. (1) There was no significant impact of postoperative adjuvant chemotherapy based on meta-analysis and previous clinical trials. (2) Confirmatory studies are necessary in large-scale prospective clinical trials. However, recent mega trials have introduced epoch-making changes for postoperative adjuvant chemotherapy in clinical practice since ASCO 2003. The effectiveness of UFT in N0 patients was confirmed. Patients with completely resected stage I non-small cell lung cancer, especially T2N0 adenocarcinoma, will benefit from adjuvant chemotherapy with UFT. The results of the International Adjuvant Lung Trial (IALT) have confirmed the meta-analysis in 1995. Also, both the JBR10 and Cancer and Leukemia Group B (CALGB) 9633 studies have also confirmed positive IALT results of the benefit for postoperative platinum-based chemotherapy in completely resected non-small cell lung cancer. Adjuvant chemotherapy for pathological stage IB to II, completely resected non-small cell lung cancer is standard care based on clinical trials. UFT showed the strongest evidence for IB in Japan. Platinum doublet chemotherapy with third-generation anticancer agents is also recommended. Adjuvant chemotherapy should be offered as standard care to patients after completely resected early stage non-small cell lung cancer. However, there is no evidence of the feasibility and efficacy for adjuvant chemotherapy with the platinum-based regimen in Japan. Careful management should be necessary in such treatment.

Key words Adjuvant therapy · Chemotherapy · Surgery · Non-small cell lung cancer · Early-stage lung cancer

Introduction

The 5-year survival rate after surgical treatment in the United States and Japan in each stage of non-small cell lung cancer is shown in Table 1.[1,2] Although these surgical outcomes reveal the slight difference among two groups, we are not satisfied with the results, particularly in stage IB and II, which are so-called early stage. Surgery is still the best therapeutic modality for the potential cure of the patient with non-small cell lung cancer, especially in stage I. However, in the patient with pathological stage IB, the 5-year survival rate is only about 60%. The recurrence pattern is frequently due to distant metastasis.[3] Therefore, perioperative adjuvant therapy is required for improvement of survival after surgical resection.

Meta-analysis of the randomized trials of adjuvant therapy of non-small cell lung cancer in 1995 suggested the survival benefit of cisplatin-based chemotherapy after surgery.[4] However, there are no statistical differences between postoperative adjuvant group and surgery alone,[4] and this includes a number of small trials and trials with the following disability criteria and chemotherapy regimens.

Consensus on adjuvant therapy for completely resected non-small cell lung cancer up to 2002 was as follows: (1) there was no significant impact of postoperative adjuvant chemotherapy based on meta-analysis and previous clinical trials. (2) Confirmatory studies are necessary in large-scale prospective clinical trials.[5–10] However, recent mega trials have introduced epoch-making changes for postoperative adjuvant chemotherapy in clinical practice since ASCO 2003.[11–19]

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Table 1. The surgical outcome for resected non-small cell lung cancer: 5-year survival rate (%)

Stage	Clinical staging		Pathological staging	
	Mountain	Japan	Mountain	Japan
IA	61	72.1	67	79.5
IB	38	49.9	57	60.1
IIA	34	48.7	55	59.9
IIB	24	40.6	39	42.2
IIIA	22	35.8	38	29.8
IIIB	9	28.0	3-7	19.3
IV	13	20.9	1	20.0

The new paradigm shift for the adjuvant treatment after surgery is demonstrated here by the Japanese and international trials that have been reported since 2003.

Japanese trials

A large-scale randomized phase III study of postoperative adjuvant chemotherapy with UFT for p-stage I adenocarcinoma: the JLCRG (Japan Lung Cancer Research Group) trial (presented in ASCO 2003[13])

The oral antimetabolite UFT is composed of tegafur and uracil mixed at the ratio of 1:4. This drug has been developed by Taiho Pharmaceutical Corporation, Tokyo, Japan. UFT produced higher levels of 5-FU without the toxic level of 5-FU.

Concerning adjuvant treatment using UFT, the West Japan Study Group for Lung Cancer Surgery reported that postoperative adjuvant treatment with UFT in patients with completely resected stage I-III disease prolonged survival significantly longer than observation alone. The 5-year and 10-year survival rates were 64% and 48% in the UFT group, and 49% and 32% in the control group, respectively ($P = 0.02$).[11] In a subgroup analysis, no statistically significant difference in the overall survival of patients with squamous cell carcinoma between the two groups was observed ($P = 0.24$). In contrast, the patients with adenocarcinoma in the UFT group had a significantly better survival than those in the control group ($P = 0.009$).[12] In addition, most patients with adenocarcinoma had stage I disease. This trial demonstrated that UFT was useful in postoperative adjuvant chemotherapy against the earlier stage of non-small cell lung cancer. However, this study involved issues with respect to study design, because enrolled subjects varied from stage I to III, with a broad range of outcome. Those results prompted us to conduct a prospective randomized trial of UFT as a postoperative adjuvant treatment for patients whose stage I adenocarcinoma was completely resected. In the confirmatory study conducted by the Japan Lung Cancer Research Group (JLCRG), patients with completely resected pathological stage I adenocarcinoma of the lung were randomized with stratification according to their

pathological T status (T1 versus T2), gender, and age, which were separated between less than 65 years old and 65 years old or over, to either receive the oral administration of UFT (tegafur 250 mg/m^2/day) for 2 years or no treatment. The patients with limited resection, such as wedge resection, were excluded. A follow-up examination was performed every 3 months for the first 2 years after the patient's operation and every 6 months thereafter. The primary endpoint was overall survival.

From January 1994, through March 1997, 999 patients were entered into the study. Twenty patients withdrew their informed consent or were found to be ineligible before the start of treatment. The number of eligible randomized patients was 491 in the UFT group and 488 in the nontreatment control group. Main patient characteristics were as follows: men, 48.7%; more than 65 years old, 43.9%; pathological T1, 73.1%. There were no significant differences in the baseline characteristics of the patients. The median duration of follow-up for all 979 patients was 73 months, with range 61–94 months.

Few severe adverse reactions were associated with UFT administration. There was no grade 4 adverse reaction. In total, 10 (2%) of 482 patients developed a grade 3 adverse reaction. The percentage of compliance for UFT administration was calculated based on the number of patients who actually took UFT and the number of patients without recurrence, second cancer, or death who were expected to take UFT. The percentage of compliance was 80% [95% confidence interval (CI), 77%–84%) at 6 months, 74% (95% CI, 70%–78%) at 12 months, 69% (95% CI, 65%–73%) at 18 months, and 61% (95% CI, 77%–84%) at 24 months. The main reasons for discontinuation of UFT administration were as follows: an adverse reaction in 123 patients, patient refusal in 52, and the doctor's judgment in 34.

Overall survival between the two groups showed a statistically significant difference in favor of the UFT group based on a Kaplan–Meier analysis ($P = 0.04$). The 5-year survival rate (5YS) was 87.9% in the UFT group and 85.4% in the control group, respectively. Treatment failure was documented in 22.6% of the patients in the UFT group and 26.4% in the control group, respectively. The most frequent failure pattern was distant metastasis in both groups. The 5-year cancer-free survival rate was 82.8% in the UFT group and 80.4% in the control group. There is no significant difference between the two groups at $P = 0.25$.

Concerning subset analysis of pathological T factors, although there was no statistical difference in the T1 population, in the T2 subset, the 5-year survival rate was 84.9% in the UFT group and 73.5% in the control group. The hazard ratio was 0.0842 in the UFT group with a clear statistical difference ($P = 0.0051$). Concerning interaction in relation to treatment effect, treatment with UFT tended to improve the survival rate among the patients with tumors that were 2–3 cm in diameter and provided 30% survival benefit for patients with tumor that was more than 3 cm in diameter. These findings indicated that the effect of UFT might be related to certain biological factors.

In conclusion, oral demonstration with UFT in the postoperative adjuvant setting yielded a significant improvement in survival in patients with pathological stage I adenocarcinoma of the lung, particularly in stage 1B, T2 N0 M0. These results of this study may be able to confirm the previous UFT adjuvant trial.

Meta-analysis of six randomized adjuvant trials with UFT (presented at ASCO 2004[14])

Clinical trials assessing the response of non-small cell lung cancer to postoperative adjuvant chemotherapy should use survival as the primary endpoint. Response should be evaluated by means of randomized controlled studies using surgical therapy alone as control. Single studies usually do not provide clear-cut conclusions because of limited sample size. A meta-analysis of all properly randomized clinical trials comparing long-term adjuvant chemotherapy with UFT, an oral fluorinated pyrimidine derivative, with surgery alone in patients with completely resected non-small cell lung cancer was demonstrated.

Six randomized trials have been conducted that compare surgery alone with adjuvant chemotherapy with UFT. The analysis was based on individual patient data provided by the principal investigator of each trial. In data from 2003, eligible patients were analyzed on an intention-to-treat bias. The endpoint of interest was overall survival at 5 years after surgery. Major prognostic factors were well balanced between the UFT group and surgery-alone group. Most patients had early-stage non-small cell lung cancer. The distribution of pathological T1 and T2 stages among this population was 65% and 34%, respectively.

The 5-year overall survival rate and 7-year overall survival rate were 81.8% and 76.5% and 77.2% in the control group; 7-year overall survival rates were 81.8% and 76.5% and 77.2% in the control group, and 7-year overall survival was 69.5% for the surgery-alone group. The result of meta-analysis demonstrated that adjuvant chemotherapy with UFT significantly improved the overall 5-year survival rate, with hazard ratio (HR) 0.77 (95% CI, 0.63–0.94; P = 0.011). Heterogeneity of effect among the six studies was not significant (P = 0.76).

The subset analysis of this meta-analysis indicated that UFT treatment provided a definitive survival benefit in most of the subset. This meta-analysis of the T1 subset population demonstrated that treatment with UFT provided a definitive survival benefit for patients with tumor that was 2–3 cm in diameter. Therefore, on the basis of our meta-analysis, postoperative adjuvant chemotherapy with UFT has a beneficial effect on outcome in patient with curatively resected non-small cell lung cancer more than 2 cm in size. Recently, Dr. Hotta from Okayama University has also demonstrated the benefit of UFT in the postoperative adjuvant setting based on the meta-analysis of five abstracts regarding UFT adjuvant trials (HR, 0.799; 95% CI, 0.668–0.957, P = 0.015).[15] These results seem to confirm the previous Hamada data.

A randomized phase III study for Bestatin (Ubenimex) as postoperative adjuvant treatment in patients with stage I squamous cell lung cancer (presented at ASCO 2001[16])

In a placebo-controlled phase III trial sponsored by the Japanese NK421 Lung Cancer Study Group, the more derived immunomodulator Bestatin (Ubenimex) was used as adjuvant therapy for patients with stage I squamous cell carcinoma following completed resection.

Confirmation of the patient eligibility and the randomization were performed within 4 weeks after each operation. The oral administration started within 1 week after their randomization. One capsule of either Bestatin or placebo was administered orally after breakfast every day for 2 years postoperatively. No additional treatment was allowed until definitive recurrence or appearance of second cancer was diagnosed.

A follow-up examination was performed every 3 months for 2 years after operation and every 6 months thereafter. The primary endpoint of the study was overall survival, and the second endpoint was disease-free survival and safe assessment. The number of patients was 202 in the Bestatin group and 198 in the placebo group. There is no significant difference in baseline characteristic of patients; 97.6% and 96.3% of the projected dose of Bestatin and placebo were administered, respectively.

The median duration follow-up for 400 patients was 77 months. Overall 5-year survival rate was significantly increased for patients receiving Bestatin compared with those receiving placebo. Disease-free survival was also significantly higher in the Bestatin group compared with placebo group, 71% versus 62%. According to multivariate analysis for survival, significant prognostic factors were performance status (PS), blood transfusion, and treatment arm.

Short summary and consideration of Japanese adjuvant trials

A couple of randomized clinical trials have demonstrated survival advantage in patients predominantly with no lymph metastasis. The effectiveness of UFT in N0 patients was confirmed. The patients with completely resected stage I non-small cell lung cancer, especially T2N0 adenocarcinoma, will benefit from adjuvant chemotherapy with UFT. UFT provides 2.5% (T2, 11.4%) benefit for absolute 5-year survival rate. HR for death in patients with stage I and T2 was 0.706 and 0.48, respectively.

Future issues for UFT adjuvant chemotherapy are to be considered as follows: (1) Do patients with stage II and/or stage III disease benefit from UFT adjuvant therapy? (2) Which regimen is better, UFT or platinum-based doublet chemotherapy in the patient with stage IB and II or III? (3) Is treatment for 1 year equivalent to treatment for 2 years? (4) What is the mechanism of UFT effectiveness in the adjuvant setting? and (5) There is need for confirmatory studies in other countries. As for Bestatin, it is necessary to do another confirmatory clinical trial.

Table 2. Potential functional mechanism of UFT and bestatin

	UFT	Bestatin
Production	5-FU derivative; tegafur and uracil	Culture filtrate of *Streptomyces olivoleticuli*
Basic concept	Antimetabolic drug	Immunomodulator
Anticancer effect (possibility)	Biochemical moduration, incidence of apotosis, inhibition of angiogenesis	Inhibition of angiogenesis Introduction of apotopsis

Table 3. The efficacy of the postoperative adjuvant chemotherapy for non-small cell lung cancer based on the pathological stage
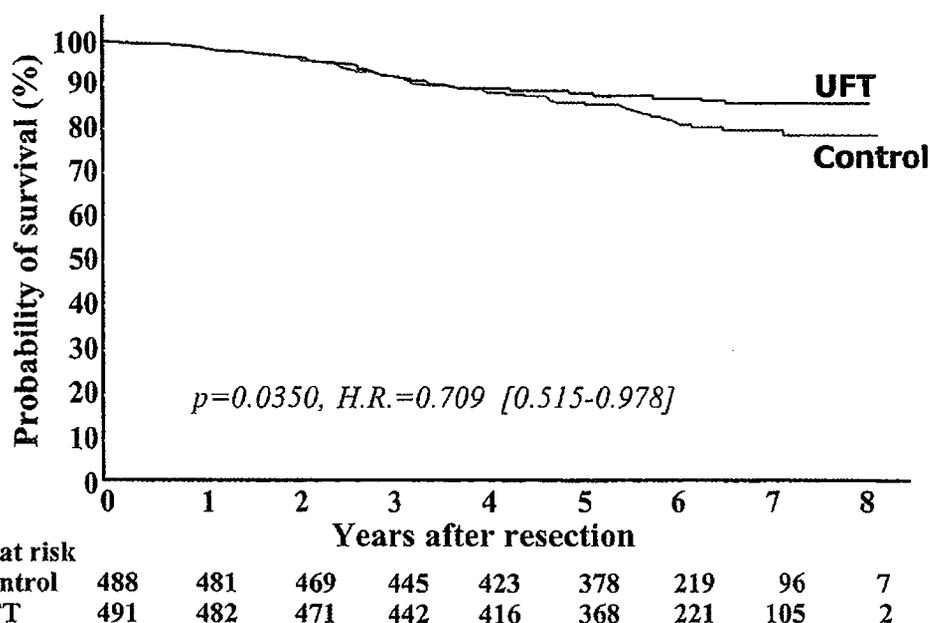
	IALT	JBR 10	CALGB 9633	JLCRG/UFT
p-stage I	Negative	Positive (IB)	Positive (IB)	Positive
p-stage II	Negative	Positive		
p-stage IIIA	Positive			
Survival benefit[a]	4.1%	15%	12% (4-year survival)	2.5% IB 11%
HR	0.86	0.70	0.62	0.71 IB 0.48
95% CI	0.76–0.98	0.52–0.92	0.41–0.95	0.51–0.98 IB 0.29–0.81

Positive, 10% improvement for the hazard ratio
HR, hazard ratio; 95% CI, 95% confidential interval
[a]Absolute difference of the 5-year survival rate between the adjuvant group and the surgery-alone group

Fig. 1. Overall survival among all 979 eligible patients in the Japan Lung Cancer Research Group (JLCRG) trial. The hazard ratios indicate the risk of death in the UFT group as compared with the control group; 95% confidential intervals are shown in *brackets*. UFT, uracil-tegafur (From ref. 13 with permission)

$p=0.0350$, $H.R.=0.709$ $[0.515-0.978]$

No. at risk	0	1	2	3	4	5	6	7	8
Control	488	481	469	445	423	378	219	96	7
UFT	491	482	471	442	416	368	221	105	2

On the basis of the comparison of mechanism between UFT and Bestatin, both drugs have been shown to inhibit angiogenesis and induce apoptosis in vivo and in vitro (Table 2). Although these data should be confirmed in future, the administration of a less cytotoxic agent and/or cytostatic drug in the adjuvant setting may improve survival for patients with early-stage non-small cell lung cancer.

Brief results of international trials

International adjuvant lung trial (IALT) (presented at ASCO 2003[17])

On the basis of a previous meta-analysis, an international adjuvant lung cancer trial was designed to evaluate the effect of cisplatin-based adjuvant chemotherapy on survival after completely resection of non-small cell lung cancer. Patients were randomly assigned either to three or four

Fig. 2. Surgical outcome of T2 subset population in the JLCRG trial. UFT, uracil-tegafur (From ref. 13 with permission)
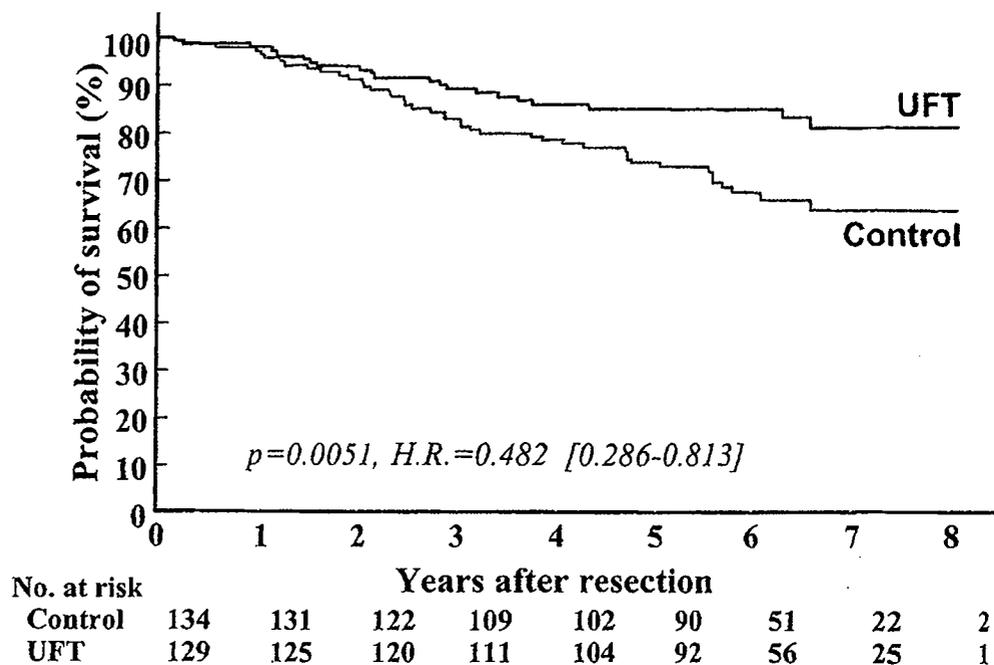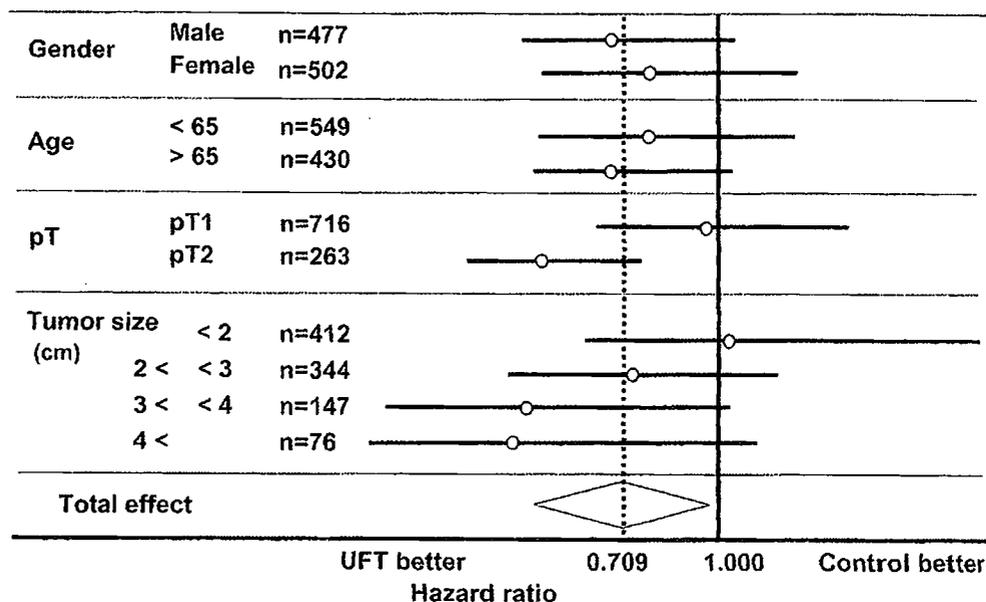

$p=0.0051,\ H.R.=0.482\ [0.286-0.813]$

No. at risk									
	0	1	2	3	4	5	6	7	8
Control	134	131	122	109	102	90	51	22	2
UFT	129	125	120	111	104	92	56	25	1

Fig. 3. Interaction in relation to treatment effect. Each *square* represents the estimated treatment effect, *horizontal lines* represent the 95% confidential intervals (CI), and the *diamond* corresponds to the 95% CI for the entire group of patients (From ref. 13 with permission)

Gender	Male	n=477
	Female	n=502
Age	< 65	n=549
	> 65	n=430
pT	pT1	n=716
	pT2	n=263
Tumor size (cm)	< 2	n=412
	2 < < 3	n=344
	3 < < 4	n=147
	4 <	n=76
Total effect		

UFT better   0.709   1.000   Control better

Hazard ratio

cycles of cisplatin-based chemotherapy or to observation (without chemotherapy). Before randomization, in each center, time in the pathological stage to be included in its policy for chemotherapy and postoperative radiotherapy policy were determined. The main endpoint was overall survival.

A total of 1867 patients underwent randomization; 36.5% had pathological stage I disease, 24.2% stage II, and 39.3% stage III. The drug allocated with cisplatin was etoposide in 56.5% of patients, vindesine in 26.8%, vinblastine in 11%, and vindesine in 5.58%. Of the 932 patients assigned to chemotherapy, 73.8% received at least 240mg

cisplatin per square meter of body surface area. In total, 23% of 932 patients developed a grade 4 adverse reaction. Seven patients (0.8%) died of chemotherapy-induced toxic effects. The median duration of follow-up was 56 months. Patients assigned to chemotherapy had a significant higher survival rate than those without chemotherapy (44.5% vs. 40.4% at 5 years; HR for death, 0.86; 95% CI, 0.76–0.93; P < 0.003). Disease-free survival rate was also significantly different between the two group (39.4% vs. 34.3% at 5 years; HR, 0.83; 95% CI, 0.74–0.94; P < 0.003). Seven patients (0.8%) died of chemotherapy-related toxic events. A total of 22.6% of the patients had at least one episode of

Fig. 4. Overall survival among all 2003 eligible patients in meta-analysis of six UFT trials. *P* values were calculated with stratified log-rank test (From ref. 14 with permission)
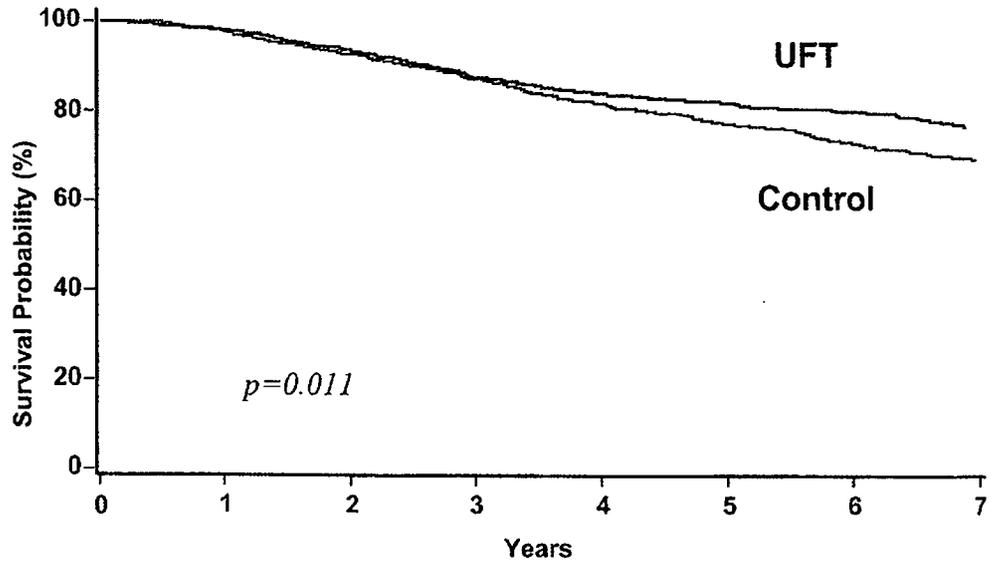

Fig. 5. Overall survival for exploratory analysis of T1 population (*n* = 1269) in UFT meta-analysis. *P* values were calculated with stratified log rank test (From ref. 14 with permission)
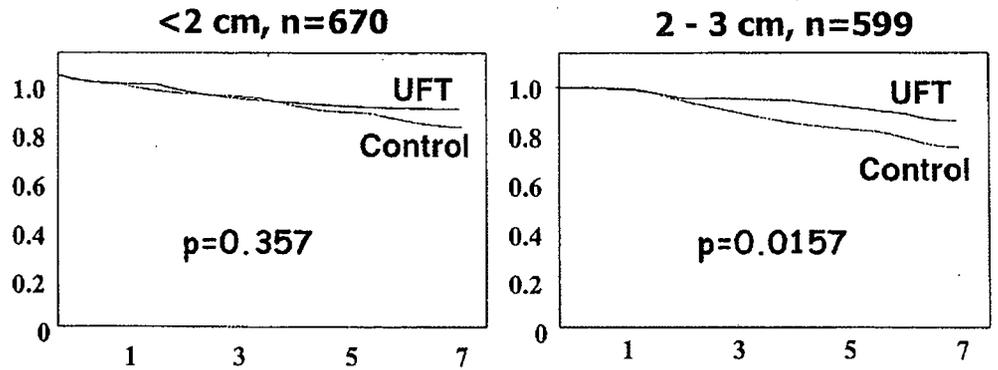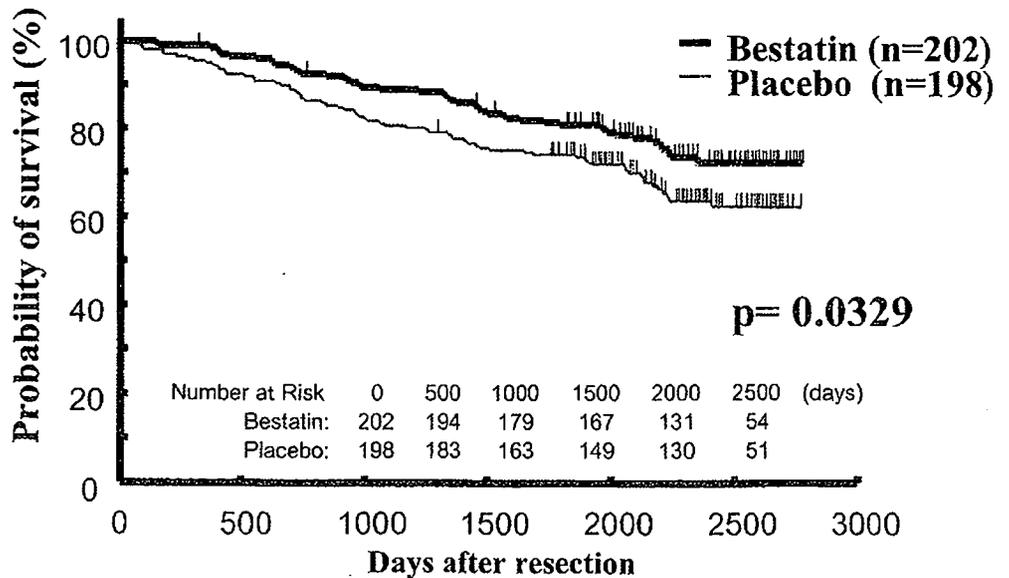

Fig. 6. Overall survival among all 400 eligible patients in Bestatin trial. *P* values were calculated with stratified log-rank test. (From ref. 16 with permission)

grade 4 toxicity, mainly neutropenia (17.5%), thrombocytopenia (2.6%), and vomiting (3.3%). These results have confirmed the meta-analysis in 1995.

NCI-Canada trial, JBR10 (presented at ASCO 2004[18])

Patients with p-stage IB and II except T3N0 were randomly assigned either to three or four cycles of cisplatin-based chemotherapy with cisplatin ($50 mg/m^2$, days 1, 8, every 4 weeks) or vinorelbine ($25 mg/m^2$, weekly to 16 weeks), or observation. A total of 344 patient underwent randomization. Stratified factors were the status of lymph node and *ras* gene. In overall survival in this study, patients with chemotherapy had a significantly higher survival rate than those with observation (69% vs. 54%, $P = 0.012$), at HR of 0.696 (95% CI, 0.524–0.923).

U.S. trial, Cancer and Leukemia Group B (CALGB) 9633 (presented in ASCO 2004[19])

Patients with p-stage IB were randomly assigned to either three or four cycles of the chemotherapy with carboplatin (AUC = 6, day 1, every 3 weeks) and paclitaxel ($200 mg/m^2$, day 1, every 3 weeks), or observation. A total of 482 patients underwent randomization. Stratified factors were histology, differentiation, and the status of mediastinoscopy. The median duration to follow-up was 34 months; patients assigned to chemotherapy had a significantly higher survival rate than those assigned to observation (71% vs. 59% at 4-year survival rate, $P = 0.028$). HR for this trial was 0.62 (95% CI, 0.41–0.95).

Short summary of international trials

The NCI-C and CALGB studies confirmed positive IALT results of the benefit for postoperative platinum-based chemotherapy in completely resected non-small cell lung cancer. The good results of NCI-C and CALGB trials might be due to patient selection, such as earlier-stage disease (IB and II), uniform patient population, more frequent incidence of women than ILT, and the therapeutic strategy of chemotherapy, such as a two-drug regimen with third-generation agent, better compliance, and no radiotherapy in patients without lymph node metastasis.

The summary was based on the international trial; consistent reductions in the risk of death have been observed in recent adjuvant platin-based trials and the 1995 meta-analysis. Adjuvant platinum-based chemotherapy should be recommended to completely resected non-small cell lung cancer patients with good performance status.

Consideration: future perspective

Even if completely resected stage I non-small cell lung cancer is due to recurrent disease in the majority of pa-

tients, adjuvant therapy had aimed at eradication of micrometastasis. Recent development of molecular biological techniques permits us to predict the chemotherapeutic response. In the adjuvant setting, the selection of anticancer drugs should depend on the analysis of molecular biological makers for resected materials in addition to pathological stage. In addition to cooperation with new chemotherapeutic agents, such as taxane, camptothecin, and gemcitabine, there are even newer classes of antineoplastic therapy, such as antiangiogenic inhibitor and tyrosine kinase inhibitor, that should be defined. The role of newer classes of some biological therapies with anticancer effect will be defined in coming years. The clinical benefit of platinum-based adjuvant therapy was confirmed. This paradigm is strongly recommended at stage IB and II non-small cell lung cancer. In stage IIIA, further subset analysis is necessary in the new meta-analysis, including IALT (Table 3). On the other hand, platinum-based chemotherapy has some potential of severe adverse events. Although there was no treatment-related death by carboplatin with paclitaxel in the CALGB trials, the feasibility of the platinum-based regimen in the adjuvant setting has not been confirmed yet in Japan. Careful observation after platinum-based chemotherapy is necessary.

Conclusion

Adjuvant chemotherapy for pathological stage IB to II, completely resected non-small cell lung cancer is standard care based on clinical trials. UFT showed the strongest evidence for IB in Japan. Platinum doublet chemotherapy with a third-generation anticancer agent is also recommended. Although there is no evidence of the feasibility of a platinum-based regimen in the adjuvant setting in Japan, adjuvant chemotherapy should be offered as standard care to patients after completely resected early-stage non-small cell lung cancer.

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Quantitative Detection of Lung Cancer Cells by Fluorescence In Situ Hybridization: Comparison With Conventional Cytology

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Quantitative Detection of Lung Cancer Cells by Fluorescence *In Situ* Hybridization*

Comparison With Conventional Cytology

Haruhiko Nakamura, MD, PhD; Idiris Aute, MD, PhD; Norihito Kawasaki, MD; Masahiko Taguchi, MD, PhD; Tatsuo Ohira, MD, PhD; and Harubumi Kato, MD, PhD, FCCP

Study objective: The aim of this study was to clarify whether fluorescence *in situ* hybridization (FISH) can diagnose lung cancer in various clinical specimens in comparison with conventional cytology.
Design: Prospective study.
Setting: University hospital in a metropolitan area.
Patients: Fifty consecutive patients with abnormal chest radiography or CT scan findings were enrolled. The patients included 32 men and 18 women, with an average age of 64 years. The final definitive diagnosis was made by histologic examination, as follows: 38 primary lung cancers (24 adenocarcinomas, 8 squamous cell carcinomas, 2 large cell carcinomas, and 4 small cell carcinomas); 1 metastatic renal cell carcinoma; and 11 benign lesions.
Methods: Four types of clinical specimens were analyzed. Cells obtained by transbronchial brushing and transbronchial fine-needle aspiration using a fiberoptic bronchoscope under fluoroscopy, CT scan-guided percutaneous needle biopsy, and bronchial washings. On every examination, duplicate slides were made for analyses of conventional cytology and FISH.
Results: Classifications according to conventional cytology were as follows: class I, 4 patients; class II, 15 patients; class IIIa, 3 patients; class IIIb, 5 patients; and class V, 23 patients. A classification higher than class IIIb was considered to be positive for cancer. For cytology, we found no false-positive cases and 11 false-negative cases. The specificity was 100%, and the sensitivity was 71.8%. By FISH, 34 cases showed aberrant copy numbers in either chromosome 3 or 17. We found no false-positive cases and five false-negative cases. The specificity was 100%, and the sensitivity was 87.1%.
Conclusion: The ability of FISH to detect aneusomic lung cancer cells is superior to conventional cytology for the diagnosis of lung cancer. *(CHEST 2005; 128:906–911)*

Key words: aneuploidy; aneusomy; cytology; fluorescence *in situ* hybridization; lung cancer

Abbreviations: BW = bronchial washing; FISH = fluorescence *in situ* hybridization; PN = percutaneous needle biopsy; SSC = standard saline citrate; TB = transbronchial brushing

C onventional cytology plays an important role for the diagnosis of lung cancer, especially in the examination of sputum and pleural effusions. In addition, cell specimens obtained by transbronchial brushing (TB)[1] and needle aspiration under fluoroscopy,[2] percutaneous needle biopsy (PN) under CT scanning,[3] and bronchial washings (BWs)[4] provide important information for the differential diagnosis between benign and malignant disease. Cytologic diagnoses are made by experienced cytologists who can properly evaluate the morphologic features of malignant cells. However, this judgment is some-

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times difficult when the morphologic changes associated with malignancy are mild. Such cells are usually classified as class III, using the classification of Papanicolaou,[5] which is suggestive of, but not conclusive for, malignancy. This is an ambiguous judgment for clinical decision making. In addition, when one obtains a small number of cells from the lesion, the definitive diagnosis is even more difficult. These limitations of morphology-based conventional cytology have stimulated the search for more objective and quantitative methods for an accurate cytologic diagnosis of cancer.

Aneuploidy is the most common feature of many solid tumors, including lung cancer.[6] Solid tumors are characterized by complicated karyotypes by classic cytogenetics.[7,8] Chromosomal instability[9,10] may cause the uneven distribution of chromosomes during cell division.[11,12] Thus, malignant tumors can be diagnosed by detecting aneuploid, usually hyperdiploid, cells. A rapid and sensitive method for detecting aneusomy of a specific chromosome in an individual cell is fluorescence in situ hybridization (FISH). For this purpose, specific centromeric DNA probes enumerated the chromosomes. FISH was originally developed as a method to detect chromosomal aberrations,[13] and is now widely used for gene mapping,[14] the diagnosis of congenital diseases,[15] and detecting specific gene copy number changes in malignant cells.[16-18]

One advantage of FISH in detecting malignant cells is its objective and quantitative evaluation. However, the specificity and sensitivity of FISH in the diagnosis of lung cancer is unclear. We report the results of a prospective study comparing FISH with conventional cytology to detect lung cancer cells.

MATERIALS AND METHODS

Patients

Fifty consecutive patients who underwent cytologic examination for abnormal chest radiography or CT scan findings at Tokyo Medical University Hospital from July 2003 to January 2004 were enrolled in this prospective study. The patients included 32 men and 18 women, with an average age of 64 years. The final definitive diagnosis was made by histologic examination, as follows: 38 primary lung cancers (24 adenocarcinomas, 8 squamous cell carcinomas, 2 large cell carcinomas, and 4 small cell carcinomas); 1 metastatic renal cell carcinoma; and 11 benign lesions. All patients with lung cancer were staged according to the latest Union Internationale Contre le Cancer criteria.[19] Cases included 10 tumors in stage IA, 5 in stage IB, 1 in stage IIA, 3 in stage IIB, 10 in stage IIIA, 6 in stage IIIB, and 3 in stage IV (Table 1).

Cells gathered from lung lesions were independently analyzed by conventional cytology and FISH. Informed consent for the cytologic examinations and genetic analyses of the specimens were obtained from all patients.

Table 1—*Histology and Stage of Lung Cancer in This Series of Patients**

Case	Age	Gender	Specimen	Histology	Stage
1	59	M	TB	Sq	cIIIA
2	42	F	PN	B	NA
3	43	M	TB	Ad	PIIIA
4	70	M	TB	Ad	CIV
5	77	M	TB	Ad	PIA
6	73	M	PN	Ad	PIA
7	58	M	TN	Ad	PIA
8	71	M	BW	Ad	pIIIA
9	71	M	BW	La	pIB
10	66	F	TN	RCC	NA
11	65	F	TB	Sm	cIIIB
12	68	F	PN	Ad	pIA
13	69	F	BW	Ad	pIV
14	52	F	TB	Ad	pIIA
15	75	F	TN	Sq	pIA
16	37	M	PN	B	NA
17	64	M	PN	Ad	pIIB
18	58	F	TB	B	NA
19	73	F	PN	Ad	pIA
20	62	M	PN	B	NA
21	69	M	TB	B	NA
22	76	M	BW	La	cIIIB
23	76	M	PN	Ad	cIIIB
24	75	M	PN	Ad	pIB
25	65	M	BW	Sm	cIIIA
26	23	M	PN	B	NA
27	74	M	BW	Ad	cIV
28	72	F	PN	B	NA
29	80	M	TB	B	NA
30	56	M	BW	Ad	pIB
31	64	M	TB	B	NA
32	58	M	PN	Ad	cIIIA
33	72	F	PN	Ad	pIA
34	72	M	PN	Ad	cIIIA
35	66	F	TB	Sm	cIIIB
36	61	M	TB	Ad	pIIB
37	72	F	TB	Ad	pIB
38	52	M	PN	B	NA
39	64	M	TB	Ad	pIA
40	79	F	TB	Sq	cIB
41	39	M	TB	B	NA
42	78	M	BW	Sq	cIIIB
43	62	M	TB	Sq	cIIIA
44	57	M	TB	Sq	pIIIA
45	70	M	PN	Ad	pIA
46	55	F	PN	Ad	pIA
47	58	F	TN	Sq	cIIIB
48	66	F	TN	Ad	pIIB
49	62	M	BW	Sm	cIIIA
50	72	F	BW	Sq	cIIIA

*M = male; F = female; TN = transbronchial needle biopsy; Ad = adenocarcinoma; Sq = squamous cell carcinoma; La = large cell carcinoma; Sm = small cell carcinoma; RCC = renal cell carcinoma; B = benign lesion; c = clinical stage; p = pathologic stage; NA = not applicable.

Cell Samples

In this study, the following four types of cell specimens were analyzed: cells obtained by TB (n = 18) and transbronchial fine-needle aspiration (n = 5) using a fiberoptic bronchoscope

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under fluoroscopy, CT scan-guided PN using the 19-gauge Tokyo Medical University Needle[3] (n = 17), and BW's (n = 10). On every examination, duplicate specimens were made for simultaneous analyses of conventional cytology and FISH.

For conventional cytology, cells were stained by the Papanicolaou method.[5] Diagnosis was made by cytologists in the Department of Pathology at Tokyo Medical University Hospital. The various classes in conventional cytology are defined as follows: class I, absence of atypical or abnormal cells; class II, atypical cytology but no evidence of malignancy; class III, cytology suggestive of, but not conclusive for, malignancy (IIIa, mild dysplasia; IIIb, advanced dysplasia); class IV, cytology strongly suggestive of malignancy; and class V, cytology conclusive for malignancy.[5]

FISH

For FISH, cells on glass slides were air-dried overnight and stored at −80°C until they were used. Direct fluorochrome-labeled centromeric probes were used for the enumeration of different chromosomes. Spectrum-orange-labeled or Spectrum-green-labeled probes for the respective centromeric regions of chromosomes 3 and 17 were purchased (Vysis Inc; Downers Grove, IL), and dual-color FISH was performed. Slides were denatured by incubation with 70% formamide (two times the standard saline citrate [SSC] solution) at 74°C for 2 min in a water bath. Then, slides were dehydrated through a graded ethanol system (70% for 2 min, 85% for 2 min, and 100% for 2 min). A hybridization solution (10 μL) was applied to each slide, which was coverslipped and sealed with rubber cement. The hybridization solution contained 1 μL each DNA probe in 70% formamide (two times the SSC solution), and 10% dextran sulfate solution (cot 1 DNA). After incubation for 16 h at 37°C in a humidified chamber, slides were washed (two times SSC solution) for 3 min at 74°C. A di-amidinophenylindole antifade solution (8 μL) was applied to each spot and coverslipped. The slides were observed under a fluorescence microscope that was connected to a cooled charge-coupled device camera and an image analyzer system (CytoVision; Applied Imaging, Ltd; Newcastle, UK).

FISH signal analysis was performed as follows. All cells in a fluorescence microscopy field, except for those with damaged or overlapped nuclei, were evaluated. One hundred cells were counted, and the numbers of each centromeric signal were recorded. If there were < 100 cells on the slide, as many cells as possible were counted. When the percentages of hyperdisomic cells (ie, more than three copies for at least one chromosome) were > 10%, we judged the lesion to be malignant.

Comparison of Conventional Cytology and FISH

FISH diagnoses were made without clinical information or the results of conventional cytology. The results of FISH analysis were not shown to the cytologists. Thus, both diagnoses were independently made in a blind fashion.

Statistical Analysis

Differences in the number of countable cells according to the histology of the lung lesions or the cell-gathering methods used were analyzed by the Kruskal-Wallis test. A p value of < 0.05 was considered to be significant.

RESULTS

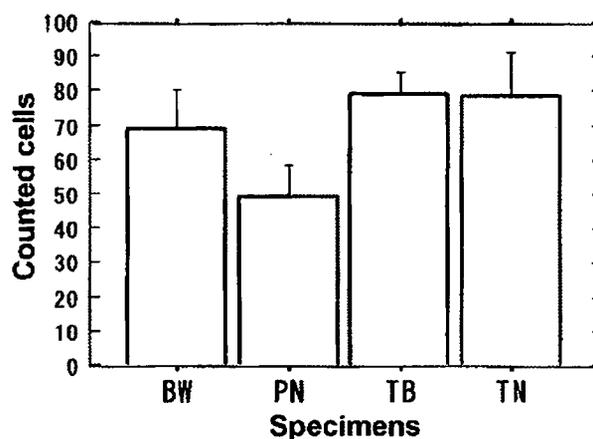Cells countable for FISH analyses ranged from 5 to 100 (maximum). Cell counts according to the

FIGURE 1. Countable cells according to the type of clinical specimen. Although cell counts obtained by PN were the lowest, no statistical significance was obtained by the Kruskal-Wallis test (p = 0.1117). Error bars indicate standard error. TN = transbronchial fine-needle aspiration.

cell-gathering method did not differ significantly, but the fewest cells were obtained by PN (Fig 1). Although the fewest cells were obtained from small cell carcinomas, no significant difference was seen according to the histologic type of lung cancer (Fig 2).

The results of conventional cytology according to the Papanicolau classification were as follows: class I, 4 cases; class II, 15 cases; class IIIa, 3 cases; class IIIb, 5 cases; and class V, 23 cases (Table 2). Twenty-eight cases showing a higher grade than class IIIb were considered to be positive for lung cancer. By cytology, we found no false-positive cases and 11
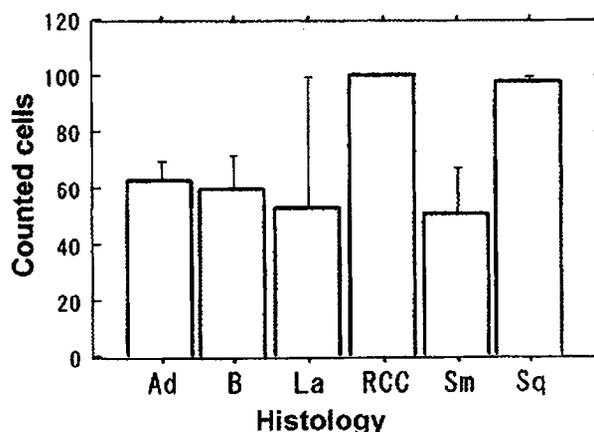

FIGURE 2. Countable cells according to histology. Although cell counts obtained from small cell lung cancer (Sm) were the lowest, no statistical significance was obtained by the Kruskal-Wallis test (p = 0.2369). Error bars indicate SE. Ad = adenocarcinoma; B = benign lesion; La = large cell carcinoma; RCC = renal cell carcinoma; Sq = squamous cell carcinoma.

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Table 2—Results of FISH and Conventional Cytology*

Case	Countable Cells	Three Copies C3/C17	Four Copies C3/C17	Five Copies C3/Ch17	Six Copies or More C3/C17	Hyperdisomy, % C3/C17	FISH	Cytology Stage
1	100	28/18	10/2	2/2	0/0	40/22	Positive	V
2	8	0/0	0/0	0/0	0/0	0/0	Negative	II
3	100	14/9	4/4	1/1	0/0	18/13	Positive	V
4	100	16/8	0/1	1/0	0/0	17/9	Positive	III
5	52	2/0	1/0	0/0	0/0	3/0	Negative†	III
6	100	9/0	1/3	1/0	0/0	10/3	Positive	IIIa†
7	44	7/3	0/0	1/0	0/0	18/7	Positive	III
8	100	6/6	0/2	2/2	0/0	8/10	Positive	V
9	5	0/0	0/0	0/0	0/0	0/0	Negative†	II
10	100	17/26	2/5	1/2	0/0	20/33	Positive	III
11	100	38/37	9/2	3/0	1/0	51/39	Positive	V
12	17	2/3	2/1	1/0	0/0	29/29	Positive	V
13	79	5/11	5/3	4/1	0/0	18/19	Positive	IIIb
14	100	7/3	0/0	0/0	1/0	8/3	Negative†	III
15	100	9/0	1/3	1/0	0/0	11/3	Positive	III
16	42	0/1	0/0	0/0	0/0	0/2	Negative	II
17	100	22/16	2/11	3/5	0/1	27/33	Positive	V
18	100	2/0	0/0	0/0	0/0	2/0	Negative	I
19	26	5/8	0/0	0/0	0/0	19/31	Positive	IIIb
20	100	1/1	0/0	0/0	0/0	1/1	Negative	II
21	100	0/0	0/0	0/0	0/0	0/0	Negative	II
22	100	17/14	3/1	2/2	1/1	23/18	Positive	IIIb
23	5	1/1	0/0	0/0	0/0	20/20	Positive	IIIb
24	100	22/23	5/7	1/3	2/1	30/34	Positive	V
25	37	13/10	1/7	1/0	0/0	41/46	Positive	V
26	8	0/0	0/0	0/0	0/0	0/0	Negative	IIIa
27	43	1/1	0/0	0/0	0/0	2/2	Negative†	II
28	100	0/0	0/0	0/0	0/0	0/0	Negative	I
29	44	0/0	0/1	0/0	0/0	0/2	Negative	II
30	100	8/6	1/1	0/1	0/0	9/8	Negative†	IIIb
31	100	2/0	0/0	0/0	0/0	2/0	Negative	II
32	8	2/3	1/1	1/0	0/0	50/50	Positive	V
33	21	4/5	1/0	0/0	0/0	24/24	Positive	V
34	57	27/31	5/2	2/1	0/1	60/61	Positive	V
35	40	10/9	2/3	4/0	0/2	40/35	Positive	V
36	100	15/15	1/4	1/1	0/0	17/20	Positive	V
37	38	2/4	0/0	0/0	0/0	5/11	Positive	V
38	23	0/1	0/0	0/0	0/0	0/4	Negative	II
39	45	9/9	1/4	2/1	0/0	27/31	Positive	V
40	79	14/12	1/1	1/0	0/0	20/16	Positive	V
41	30	0/1	0/0	0/0	0/0	0/3	Negative	II
42	100	17/6	3/1	0/1	0/0	20/8	Positive	V
43	100	19/18	3/6	4/1	2/0	28/25	Positive	III
44	100	18/15	1/5	0/1	0/0	19/21	Positive	V
45	100	11/7	0/1	0/0	0/0	11/8	Positive	V
46	14	4/3	0/0	0/0	0/0	29/21	Positive	V
47	100	15/15	0/8	0/1	0/0	15/24	Positive	V
48	48	8/6	0/1	0/0	0/0	17/15	Positive	V
49	25	4/6	1/0	0/0	0/0	20/24	Positive	V
50	100	21/28	5/5	1/1	0/0	27/34	Positive	IIIa†

*C3 = chromosome 3; C17 = chromosome 17.
†False-negative result.

false-negative cases. Thus, by conventional cytology, specificity was 100%, and sensitivity was 71.8%.

In FISH analyses, 34 cases showed aberrant copy numbers in either chromosome 3 or 17. Representative findings of conventional cytology and FISH

are shown in Figure 3. We found no false-positive cases and five false-negative cases. For FISH analyses, specificity was 100%, and sensitivity was 87.1%.

Seven cases, including one with metastatic renal cell carcinoma, had negative cytology findings and

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