16. Garipagaoglu M, Tulunay G, Kose MF, et al. (1998) Prognostic factors in stage IB-IIA cervical carcinomas treated with postoperative radiotherapy. Eur J Gynaecol Oncol 131–135
17. Uno T, Ito H, Itami J, et al. (2000) Postoperative radiation therapy for stage IB-IIB carcinoma of the cervix with poor prognostic factors. Anticancer Res 20:2235–2240
18. Aoki Y, Sasaki M, Sato T, et al. (2000) High-risk group in node-positive patients with stage IB, IIA and IIB cervical carcinoma after radical hysterectomy and postoperative pelvic irradiation. Gynecol Oncol 77:305–309
19. Kodaira T, Fuwa N, Nakanishi T, et al. (2002) Retrospective analysis of postoperative radiotherapy for node-negative cervical carcinoma with stage IB-IIB disease. Jpn J Clin Oncol 32:255–261
20. Kim RY, Salter MM, Shingleton HM (1988) Adjuvant postoperative radiation therapy following radical hysterectomy in stage IB ca of the cervix. Analysis of treatment failure. Int J Radiat Oncol Biol Phys 14:445–449
21. Monk BJ, Cha DS, Walker JL, et al. (1994) Extent of disease as an indication for pelvic radiation following radical hysterectomy and bilateral pelvic lymph node dissection in the treatment of stage IB and IIA cervical carcinoma. Gynecol Oncol 54:4–9
22. Burghardt E, Pickel H (1978) Local spread and lymph node involvement in cervical cancer. Obstet Gynecol 52:138–145
23. Dargent D, Frobert, JL, Beau G (1985) V Factor (tumor volume) and T factor (FIGO classification) in the assessment of cervix cancer prognosis: the risk of lymph node spread. Gynecol Oncol 22:15–22
24. Kaplan EL, Meier P (1958) Non-parametric estimation from incomplete observation. J Am Stat Assoc 53:457–481

SPECIFIC DETECTION OF TUBERCULOSIS INFECTION: AN INTERFERON-γ-BASED ASSAY USING NEW ANTIGENS

TORU MORI, MITSUNORI SAKATANI, FUMIO YAMAGISHI, TETSUYA TAKASHIMA, YOSHIKO KAWABE, KEIJI NAGAO, ERIKO SHIGETO, NOBUYUKI HARADA, SATOSHI MITARAI, MASAJI OKADA, KATSUHIRO SUZUKI, YOSHIKAZU INOUE, KAZUNARI TSUYUGUCHI, YUKA SASAKI, GERALD H. MAZUREK, and IZUO TSUYUGUCHI

Reprinted From: American Journal of Respiratory and Critical Care Medicine
Volume 170 Number 1 July 1, 2004

Specific Detection of Tuberculosis Infection

An Interferon-γ–based Assay Using New Antigens

Toru Mori, Mitsunori Sakatani, Fumio Yamagishi, Tetsuya Takashima, Yoshiko Kawabe, Keiji Nagao, Eriko Shigeto, Nobuyuki Harada, Satoshi Mitarai, Masaji Okada, Katsuhiro Suzuki, Yoshikazu Inoue, Kazunari Tsuyuguchi, Yuka Sasaki, Gerald H. Mazurek, and Izuo Tsuyuguchi

Research Institute of Tuberculosis and National Tokyo Hospital, Tokyo; National Kinki Chuo Hospital for Chest Diseases and Osaka Prefectural Habikino Hospital, Osaka; National Chiba Higashi Hospital and Chiba University School of Medicine, Chiba; and National Hiroshima Hospital, Hiroshima, Japan; and Centers for Disease Control and Prevention and Emory University School of Medicine, Atlanta, Georgia

The tuberculin skin test for immunologic diagnosis of *Mycobacterium tuberculosis* infection has many limitations, including being confounded by bacillus Calmette-Guérin (BCG) vaccination or exposure to nontuberculous mycobacteria. *M. tuberculosis*–specific antigens that are absent from BCG and most nontuberculous mycobacteria have been identified. We examined the use of two of these antigens, CFP-10 and ESAT-6, in a whole blood IFN-γ assay as a diagnostic test for tuberculosis in BCG-vaccinated individuals. Because of the lack of an accurate standard with which to compare new tests for *M. tuberculosis* infection, specificity of the whole blood IFN-γ assay was estimated on the basis of data from people with no identified risk for *M. tuberculosis* exposure (216 BCG-vaccinated Japanese adults) and sensitivity was estimated on the basis of data from 118 patients with culture-confirmed *M. tuberculosis* infection who had received less than 1 week of treatment. Using a combination of CFP-10 and ESAT-6 responses, the specificity of the test for the low-risk group was 98.1% and the sensitivity for patients with *M. tuberculosis* infection was 89.0%. The results demonstrate that the whole blood IFN-γ assay using CFP-10 and ESAT-6 was highly specific and sensitive for *M. tuberculosis* infection and was unaffected by BCG vaccination status.

Keywords: bacillus Calmette-Guérin; diagnostics; infection; IFN-γ; tuberculosis

Tuberculosis continues to be a heavy burden on human health, with the World Health Organization estimating that one-third of the world's population is infected with *Mycobacterium tuberculosis* (1). Detection and treatment of latent tuberculosis infection are important measures in the fight against this epidemic, especially in industrialized countries. The tuberculin skin test (TST) has been the only practical means of detecting latent *M. tuberculosis* infection in the past century. Unfortunately, the TST suffers from a number of well-documented performance and logistic problems, the most serious being false-positive responses due to reactivity caused either by infection with nontuberculous mycobacteria (NTM), or by bacillus Calmette-Guérin (BCG) vaccination (2, 3).

An *in vitro* whole blood test that detects *M. tuberculosis* infection by measuring IFN-γ responses to tuberculin purified protein derivative (PPD) was approved in the United States. Although this assay may be less affected by BCG vaccination than the TST (3), it is falsely positive in some BCG-vaccinated individuals (4) as many PPD antigens are similar or identical to antigens in BCG and NTM. Parts of the *M. tuberculosis* genome that are absent from the genomes of all BCG substrains and most NTM have been identified (5). These *M. tuberculosis*–specific regions encode a number of proteins including CFP-10 and ESAT-6. Cell-mediated responses to these antigens have been shown to correlate with both proven *M. tuberculosis* infection and a high risk of infection (4, 5–10). The application of CFP-10 and ESAT-6 to the whole blood IFN-γ assay should allow specific and sensitive diagnosis of *M. tuberculosis* infection in a relatively simple test format.

Thus, the aim of this study was to estimate the specificity and sensitivity of a whole blood IFN-γ assay employing CFP-10 and ESAT-6, for the detection of *M. tuberculosis* infection in a predominantly BCG-vaccinated population. Estimates of sensitivity and specificity of tests for *M. tuberculosis* infection are hampered by the lack of a "gold standard"; one cannot prove the presence or absence of latent tuberculosis (TB) infection. In this study, sensitivity was determined in untreated patients with culture-proven tuberculosis, which although definitive for active tuberculosis requires extrapolation to equate to latent tuberculosis infection. Specificity was estimated in a group of BCG-vaccinated individuals with no known risks for *M. tuberculosis* exposure.

METHODS

Participants

Patients and student nurses consenting to the study were enrolled in Tokyo (National Tokyo Hospital, Fukujuji Hospital, and Japan Anti-Tuberculosis Association), Osaka (National Kinki Chuo Hospital and Osaka Prefectural Habikino Hospital), Chiba (National Chiba Higashi Hospital; and Nursing College, Chiba University), Miyazaki (Miyazaki Prefectural Nursing University), and Hiroshima (National Hiroshima Hospital), Japan after the protocol was approved by each institution's ethics review committee. Subjects were enrolled into one of two groups: Group 1 consisted of student nurses (older than 17 years of age) who were enrolled at the beginning of their training and had no identified risk for *M. tuberculosis* exposure; and Group 2 consisted of patients clinically suspected to have active tuberculosis and who had received less than 1 week of antituberculosis treatment.

After giving written consent, subjects were asked to complete a questionnaire about possible risk factors for exposure to *M. tuberculosis*. For low-risk subjects enrolled into Group 1, data were collected on their country of birth, history of prior tuberculosis or exposure to a person with tuberculosis, and other tuberculosis risk factors such as having an immunosuppressive condition (i.e., human immunodeficiency virus [HIV], leukemia, lymphoma, diabetes mellitus, or renal failure) or having taken immune suppressive drugs in the 3 months before enrollment. Information regarding any previous Mantoux TST results and BCG vaccination status was also collected. For patients recruited into Group 2, information on their clinical symptoms of active tuberculosis and chest

(Received in original form February 10, 2004; accepted in final form March 26, 2004)

Supported by the Research Project of Emerging and Re-emerging Diseases, Ministry of Health, Labor, and Welfare, Japan (a Study for the Development of New Tuberculosis Control Strategy); Nichirei Corporation, Tokyo, Japan; and Cellestis R&D Pty. Ltd., Melbourne, Australia.

Correspondence and requests for reprints should be addressed to Toru Mori, M.D., Ph.D., Research Institute of Tuberculosis, 3-1-24 Matsuyama, Kiyose, Tokyo 204-8533, Japan. E-mail: tmori@jata.or.jp

Am J Respir Crit Care Med Vol 170. pp 59–64, 2004
Originally Published in Press as DOI: 10.1164/rccm.200402-179OC on April 1, 2004
Internet address: www.atsjournals.org

X-ray findings were collected at the time of enrollment. Sputum or other appropriate nonrespiratory samples were collected from Group 2 patients and cultured for mycobacteria.

Sample Collection and TST

A heparinized blood sample was collected for the whole blood IFN-γ assay from each subject by venipuncture. Blood was collected before administration of Mantoux TSTs when the latter test was performed. For the TST, 0.1 ml of tuberculin PPD (Nippon BCG Manufacturing, Tokyo, Japan; equivalent to about 3 TU of PPD-S) was injected intradermally into the volar aspect of the forearm and transverse induration diameter was measured 48 hours later.

M. tuberculosis–specific Antigens

Pools of overlapping peptides representing CFP-10 and ESAT-6 were used as TB-specific antigens in the whole blood IFN-γ assay. The sequence of six peptides representing CFP-10 and of seven peptides representing ESAT-6 are shown in Table 1. Peptides, manufactured by either Mimotopes (Clayton, Australia) or Schafer-N (Copenhagen, Denmark), were at least 79% pure as determined by HPLC analysis. Peptides were solubilized in phosphate-buffered saline and aliquots (10 μg/ml for each peptide) were stored at −70°C, before use in the whole blood IFN-γ assay.

Whole Blood IFN-γ Assay

The whole blood IFN-γ assay (QuantiFERON [QFT]; Cellestis, Carnegie, Australia) involves two stages: (*1*) overnight incubation of whole blood with antigens and (*2*) measurement of IFN-γ production in harvested plasma samples by ELISA. Within 12 hours of collection, 1-ml aliquots of blood samples were dispensed into 24-well tissue culture plates and antigens were added to appropriate wells. Three drops of saline (nil control) or phytohemagglutinin (5 μg/ml; mitogen-positive control), and 100 μl of ESAT-6 or CFP-10 peptide cocktail, were added to separate wells to give a final peptide concentration of 1 μg/ml. Blood samples were incubated with antigens for 16 to 24 hours at 37°C before harvesting about 300 μl of plasma from above the settled blood cells.

The concentration of IFN-γ in the four plasma samples from each subject was determined by QuantiFERON-CMI ELISA as per the manufacturer's instructions. This ELISA is reported by the manufacturer to have a limit of detection of 0.05 IU/ml for IFN-γ. Samples from up to 16 subjects were tested in each ELISA run, which also included a set of standards that were measured in duplicate. For an ELISA run to be valid, strict performance criteria (coefficient of variation less than 15% and correlation coefficient for the standard curve greater than 0.98) had to be met. ELISA data for the *M. tuberculosis*–specific antigens CFP-10 and ESAT-6 and the nil and mitogen controls were converted to international units per milliliter on the basis of the IFN-γ standard curve generated for each ELISA plate. For an individual's test to be deemed valid, their response to at least one antigen (ESAT-6, CFP-10, or mitogen) had to be at least 0.25 IU of IFN-γ per milliliter above that of their nil control (five times the limit of detection for the ELISA). Results for ESAT-6 and CFP-10 are expressed as the concentration of IFN-γ detected minus the concentration of IFN-γ in the respective nil control plasma.

TABLE 1. AMINO ACID SEQUENCES OF OVERLAPPING PEPTIDES FOR ESAT-6 AND CFP-10

Antigen	Amino Acid Sequence
CFP-10	
Peptide 1	MAEMKTDAATLAQEAGNFERISGDL
Peptide 2	GNFERISGDLKTQIDQVESTAGSLQ
Peptide 3	DQVESTAGSLQGQWRGAAGTAAQAAV
Peptide 4	AAGTAAQAAVVRFQEAANKQKQELD
Peptide 5	AANKQKQELDEISTNIRQAGVQYSR
Peptide 6	IRQAGVQYSRADEEQQQALSSQMGF
ESAT-6	
Peptide 1	MTEQQWNFAGIEAAASAIQG
Peptide 2	GIEAAASAIQGNVTSI
Peptide 3	SAIQGNVTSIHSLLDEGKQSLTKLA
Peptide 4	EGKQSLTKLAAAWGGSGSEAYQGVQ
Peptide 5	SGSEAYQGVQQKWDATATELNNALQ
Peptide 6	TATELNNALQNLARTISEAGQAMAS
Peptide 7	NLARTISEAGQAMASTEGNVTGMFA

Statistical Analysis

Information from the questionnaires, TST results, and whole blood IFN-γ assay results was entered into Excel 2000 (Microsoft, Redmond, WA) and transferred to Stata version 7.0 (Stata, College Station, TX) for statistical analysis. Analysis consisted of *t* tests for differences in means based on logarithmic transformation of the IFN-γ measurements, χ^2 test for testing difference in proportions, exact binomial methods to compute confidence intervals for proportions, and maximum-likelihood logistic regression to estimate the strength of the relation between age and response to the whole blood IFN-γ assay and the TST.

RESULTS

Subjects were enrolled into the study over a 4-month period from July to October 2002. There were 216 people with no identified risk for *M. tuberculosis* exposure enrolled into Group 1 and 152 tuberculosis suspects enrolled into Group 2. The mean age for Group 1 subjects was 20 years (range, 18–33 years) and for Group 2, 54 years (range, 13–86 years; age was not recorded for eight people). Group 1 subjects were predominantly female (92.7%), whereas Group 2 subjects were predominantly male (66.4%). No subjects in Group 1 reported any history of contact with patients with tuberculosis or of working in any health care setting.

The majority of Group 1 subjects had last been screened with the TST when entering junior high school, 6 years before the current study. None of these subjects reported having an immunosuppressive condition such as HIV, leukemia, lymphoma, diabetes mellitus, or renal failure; and none reported having taken immune-suppressive drugs in the 3 months before enrollment. TST results were available for 113 of the 216 Group 1 subjects; of them, 97 (85.8%) had an induration 5 mm or more, 73 (64.6%) had an induration 10 mm or more, and 36 (31.9%) had an induration 15 mm or more. Thus, taking 10-mm induration as the cutoff, the specificity of tuberculin skin testing was 35.4%. The mean age and its standard error of those without TST were 19.5 years and 0.266, which compared with those with TST (19.2 and 0.238, respectively). All Group 1 subjects reported having received BCG vaccination at least once by the time of graduation from junior high school.

Of the 152 TB suspects in Group 2, 119 were proven to have *M. tuberculosis* infection (and active tuberculosis) by culture of the organism from sputum or other bodily samples. Sputum acid-fast smear results were available for only 78 of the 119 persons with culture-proven tuberculosis, as one hospital did not report smear results. Sixty-eight of 78 patients had positive smears. One person, whose culture was positive for *M. tuberculosis*, had an indeterminate QFT result due to insufficient IFN-γ production in response to the mitogen or TB-specific antigens. Results from this person were omitted from further analysis. *M. tuberculosis* was recovered from pleural fluid of four Group 2 subjects and from sputum of 114 subjects. All TB suspects had received less than 7 days of antituberculous chemotherapy at the time of testing; 95 (80.5%) had received none. TST results were available for 76 of the 118 evaluable Group 2 subjects; 50 of these (65.8%) displayed an induration of 5 mm or greater. The patients who had TST results had a mean age (± standard error) of 54.7 ± 2.3 years, compared with 51.7 ± 3.6 years for those in whom skin tests were not performed ($p = 0.74$). Both groups had a similar sex distribution (65 and 66% males, respectively; $p = 0.96$) and a similar percentage of patients with positive sputum acid-fast smears (92 and 82%, respectively; $p = 0.17$).

No patients self-reported to be seropositive for HIV, undergoing hemodialysis, currently being treated with corticosteroids, or known to have a malignant disease. There were four patients with diabetes mellitus. There were 33 people in Group 2 whose cultures were negative for *M. tuberculosis* despite symptoms and suspicion of active tuberculosis; *Mycobacterium avium* complex (MAC) organisms were recovered from 5 of these people; *Mycobacterium kansasii* was recovered from 3; and 25 had negative culture results for mycobacteria.

Response to Specific Antigens

All IFN-γ ELISA runs met the specified performance criteria and were deemed valid. The range of responses in the whole blood IFN-γ assay for subjects in each study group are shown in Figure 1. Patients with culture-proven tuberculosis had a significantly higher mean IFN-γ response than did low-risk Group 1 subjects for both CFP-10 (geometric means being 0.657 and 0.010 IU/ml, respectively; $p < 0.001$) and ESAT-6 (1.330 and 0.003 IU/ml, respectively; $p < 0.001$).

Table 2 shows test specificities and sensitivities for CFP-10 and ESAT-6 at various cutoff concentrations. To estimate specificity, all 216 subjects in Group 1 were assumed not to be infected with *M. tuberculosis*. To estimate sensitivity, only QFT results from the 118 Group 2 subjects for whom *M. tuberculosis* infection was confirmed by culture were used. To ascertain appropriate cutoffs for the ESAT-6 and CFP-10 antigens, receiver operating characteristic analysis was performed, based on data from Group 1 individuals for specificity and Group 2 patients with culture-confirmed *M. tuberculosis* infection for sensitivity. Receiver operating characteristic analysis was performed with data from these subjects and confirmed that 0.35 IU/ml was an appropriate cutoff for both CFP-10 and ESAT-6. This cutoff was chosen to maximize specificity without significant loss of test sensitivity. Using this cutoff, the specificities (with 95% confidence intervals) for CFP-10 and ESAT-6 were 98.6% (96.0 to 99.7%; n = 213, data for CFP-10 were unavailable for three people because of insufficient blood being collected) and 99.5% (97.5 to 100.0%; n = 216), respectively, and the sensitivities were 65.3% (55.9 to 73.8%) and 81.4% (73.1 to 87.9%), respectively. If the data from CFP-10 and ESAT-6 were combined such that a person positive to at least one of the two antigens is judged as test positive, a sensitivity of 89.0% (81.9 to 94.0%) and a specificity of 98.1% (95.3 to 99.5%; n = 213) were obtained.

Test results were positive in 60 (88%) of 68 patients with positive sputum acid-fast smears and 6 (60%) of 10 patients with negative smears ($p = 0.07$).

Data for the 33 people in Group 2 whose cultures were negative for *M. tuberculosis* despite symptoms and suspicion of active tuberculosis are shown in Figure 1C. For the 25 tuberculosis suspects from whom mycobacteria were not recovered, 56% (14) were positive to either CFP-10 or ESAT-6 in the whole blood IFN-γ assay, a significantly smaller proportion as compared with those with culture-confirmed *M. tuberculosis* infection (89%; χ^2 test, $p = 0.0001$). The whole blood IFN-γ assay with either antigen was positive for all three patients from whom *M. kansasii* was recovered. For one of the five patients from whom MAC was recovered, the CFP-10 response was positive (IFN-γ, 7.5 IU/ml).

To examine the effect of age on sensitivity of the whole blood IFN-γ assay and the TST, data from the 110 patients with confirmed tuberculosis, and whose ages were recorded, were stratified as shown in Table 3. Logistic regression analyses were used to estimate the associations between age and QFT response, and between age and TST response. On average, persons were 0.83 times as likely to have a positive QFT and 0.71 times as likely to have a positive TST, compared with persons 10 years younger. The 95% confidence interval for the former odds ratio was 0.56 to 1.23, with decline being not statistically significant ($p = 0.35$), and that for the latter was 0.53 to 0.94, with a statistically significant decline ($p = 0.015$).

DISCUSSION

The current study demonstrates a high degree of accuracy in detecting *M. tuberculosis* infection, using the whole blood IFN-γ assay with the *M. tuberculosis*–specific proteins CFP-10 and ESAT-6. The assay was shown to be highly specific (greater than 98%) in BCG-vaccinated low-risk subjects (Group 1) assumed to be truly free of *M. tuberculosis* infection. Specificity of the whole blood IFN-γ assay was much better than the specificity observed for the TST in the present study (35.4%, using a 10-mm induration cutoff), or previously reported for Japan (10%) (11). Although we assumed that none of the Group 1 subjects were infected with *M. tuberculosis*, it is probable that some of the 216 subjects had been infected, as the prevalence of *M. tuberculosis* infection in 20-year-old people in Japan is estimated at 1% (12).

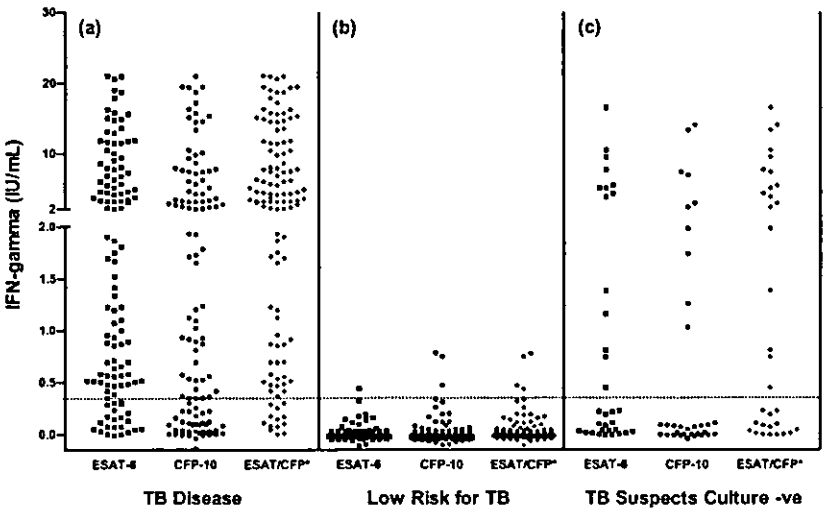

Figure 1. Dot plot of individual responses to CFP-10 and ESAT-6 for 118 culture-positive patients with tuberculosis (TB) (*a*), 213 subjects with a low risk for TB exposure (*b*), and 33 TB suspects whose TB status could not be determined, as *Mycobacterium tuberculosis* could not be cultured (*c*). *For "ESAT/CFP" the data for the antigen (ESAT-6 or CFP-10) giving the highest response is shown. The *dashed line* represents the cutoff of 0.35 IU/ml for IFN-γ.

TABLE 2. TEST SENSITIVITY AND SPECIFICITY FOR CFP-10 AND ESAT-6 AT VARIOUS CUTOFFS IN WHOLE-BLOOD IFN-γ ASSAY

Cutoff, IFN-γ	CFP-10		ESAT-6		CFP-10 and/or ESAT-6	
(*IU/ml*)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)
0.05	92.5	81.4	94.8	94.9	89.4	97.5
0.10	94.4	77.1	96.2	90.7	92.0	95.8
0.15	95.8	72.9	97.6	88.1	93.9	93.2
0.20	96.7	71.2	99.1	86.4	96.2	91.5
0.25	97.2	67.8	99.1	84.7	96.7	91.5
0.30	97.7	66.9	99.1	83.1	97.2	89.8
0.35	**98.6**	**65.3**	**99.5**	**81.4**	**98.1**	**89.0**
0.40	98.6	61.9	99.5	79.7	98.1	88.1
0.45	98.6	60.2	100.0	78.8	98.6	86.4
0.50	99.1	60.2	100.0	75.4	99.1	83.9

Sensitivity was determined on the basis of data from 118 patients with culture-positive tuberculosis, and specificity was determined on the basis of data from 213 low-risk subjects. The chosen cutoff (0.35) is in boldface.

Thus, the true specificity of the test may be higher than that estimated in the present study.

To estimate sensitivity of the whole blood IFN-γ test, the presence of culture-confirmed *M. tuberculosis* infection was used as the standard. This approach has been widely used in sensitivity studies with the TST, often using patients who were receiving, or who had completed, treatment at the time of testing (3, 13–16). However, as it is well documented that both IFN-γ responses can vary in relation to antituberculosis treatment (3, 17–19), we limited this study to patients who had received minimal or no treatment at the time of testing. At the time of enrollment into the study, all 152 Group 2 subjects had radiologic and/or clinical signs suggesting tuberculosis and sensitivity was estimated from the 118 who had *M. tuberculosis* recovered subsequently by culture. Both ESAT-6 and CFP-10 demonstrated high positive rates in these patients (65.3 and 81.4%, respectively) as compared with that in tuberculin skin testing (65.8%). Combining results from the *M. tuberculosis*-specific antigens improved test sensitivity to 89.0% and had little effect on specificity (98.1%).

The poor skin test specificity of TST (35.4%) seen in this study is likely to be predominantly a result of the extensive use of BCG vaccination in Japan. However, poor skin test specificity may also be due to exposure or infection with NTM. Exposure to NTM, and not latent *M. tuberculosis* infection, appears to be responsible for the majority of 5- to 14-mm Mantoux test reactions among U.S.-born health care workers and medical students (20). The present study was not designed to assess the specificity of the whole blood IFN-γ assay after exposure to NTM. However, given the reported mycobacterial species specificity of ESAT-6 and CFP-10 (5), the assay is likely to be negative for infection with *M. avium* complex (MAC), which is a major source of NTM infection. This was compatible with the study's finding that IFN-γ response to both of ESAT-6 and CFP-10 was negative in all patients who were culture negative for *M. tuberculosis* and positive for MAC, except one. The latter MAC patient with a positive IFN-γ response could have coinfection with tuberculosis. On the other hand, positive reactions are expected from people infected with *M. kansasii, Mycobacterium marinum,* or *Mycobacterium szulgai* as the genes encoding both ESAT-6 and CFP-10 are present in these NTM (7). Therefore, it is not surprising that another three TB suspects positive for *M. kansasii* responded to ESAT-6 and/or CFP-10 in the whole blood IFN-γ assay.

It remains to be confirmed whether the enhanced sensitivity of the whole blood IFN-γ assay over the TST, as seen for untreated patients in this study, will also be found for people with latent tuberculosis infection. However, such a possibility can be supported by reports that contacts of patients with tuberculosis, who are possibly latently infected with *M. tuberculosis*, have stronger IFN-γ responses to *M. tuberculosis* antigens than do patients with active tuberculosis (18, 19, 21–23). Further investigations on the performance of the CFP-10/ESAT-6-based whole blood IFN-γ assay in contact investigations and in other situations where *M. tuberculosis* exposure can be quantified are required to further estimate the test performance for detecting latent tuberculosis infection.

Screening for latent tuberculosis infection is most effective if those with positive test results are likely to progress to clinical disease. A preliminary study by Doherty and coworkers (24) demonstrated a close relationship between IFN-γ responses and subsequent development of clinical tuberculosis disease in household tuberculosis contacts in Ethiopia, but this needs corroboration in

TABLE 3. CFP-10 AND ESAT-6 IFN-γ ASSAY AND MANTOUX TUBERCULIN SKIN TEST RESULTS, STRATIFIED BY AGE, FOR 110 PATIENTS WITH CULTURE-POSITIVE TUBERCULOSIS

Age (*yr*)	No. IFN-γ–tested	No. IFN-γ–positive	Percent IFN-γ–positive	No. Mantoux-tested	No. Mantoux-positive	Percent Mantoux-positive
13–30	19	17	89.5	9	9	100.0
31–40	14	14	100.0	12	7	58.3
41–50	16	15	93.8	12	9	75.0
51–60	19	19	100.0	10	5	50.0
61–70	19	17	89.5	12	9	75.0
71–80	13	12	92.3	11	6	54.5
> 80	10	8	80.0	6	1	16.7

Results for the Mantoux test are based on a 5-mm cutoff.

other populations of different immune status and background. In addition, although the current study indicates utility of the IFN-γ assay in screening adults for TB infection, further studies are required, including those in select patient populations such as children, people with X-ray evidence of prior tuberculosis, and those with HIV infection or other immunodeficiencies. Test utility would also be enhanced by studies determining the kinetics of IFN-γ response after infection, and the effect of antituberculosis therapy on IFN-γ test results.

Previous studies have demonstrated the potential of both ESAT-6 and CFP-10 for the specific detection of *M. tuberculosis* infection in humans (4, 5–10), although the method generally used to measure IFN-γ responses to these antigens, such as lymphocyte proliferation and IFN-γ enzyme-linked immunospot, are relatively complex and labor intensive to perform (25). Some of these studies have demonstrated that a combination of results from ESAT-6 and CFP-10 provides higher sensitivity than is seen with either antigen alone (7, 8). In addition, Vordermeier and coworkers demonstrated greater sensitivity with a cocktail of CFP-10 and ESAT-6 over either antigen alone, when used in an IFN-γ enzyme-linked immunospot assay (26), and Arend and coworkers showed that use of both antigens increased test sensitivity, as there were variations in responses to CFP-10 and ESAT-6 between individuals with different HLA-DR types (10). These data suggest that the combined use of both TB-specific antigens is warranted to increase sensitivity and our results support this conclusion.

In addition to the high diagnostic accuracy resulting from the use of *M. tuberculosis*–specific antigens, the whole blood IFN-γ assay offers many methodologic and logistic advantages, both over the TST and other laboratory methods of immunological testing. The test requires a single patient visit, does not induce boosting of subsequent test results, and can provide results within 1 day. Interreader variability is low and results are highly reproducible (27) as it is a controlled laboratory assay. Importantly, whole blood testing uses minimal labor and simple equipment, allowing large numbers of samples to be tested concurrently.

Conflict of Interest Statement: T.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; F.Y. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; T.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; Y.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; K.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; E.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; N.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.O. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; K.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; Y.I. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; K.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; Y.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; G.H.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; I.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgment: The authors acknowledge the following people for technical assistance and input in this trial: Dr. Takashi Kitoh (Nichirei Corporation, Tokyo, Japan), Dr. Peter Andersen (Statens Serum Institut, Copenhagen, Denmark), Drs. Angela Cosgriff and Jim Rothel (Cellestis Limited, Carnegie, Australia), Dr. K. Higuchi and Ms. S. Sekiya (Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Japan), and Ms. N. Matsumoto (Miyazaki Prefectural Nursing University, Japan). The authors also thank Professor Damien Jolley for advice on statistical analysis of the data. Finally, the authors thank the participants who made this study possible.

References

1. World Health Organization. Global tuberculosis control: surveillance, planning, financing [WHO report]. Geneva, Switzerland: World Health Organization; 2002.
2. Centers for Disease Control and Prevention. Targeted tuberculin testing and treatment of latent tuberculosis infection. *MMWR Morb Mortal Wkly Rep* 2000;49:1–51.
3. Mazurek GH, LoBue PA, Daley CL, Bernardo J, Lardizabal AA, Bishai WR, Iademarco MF, Rothel JS. Comparison of a whole-blood interferon γ assay with tuberculin skin testing for detecting latent *Mycobacterium tuberculosis* infection. *JAMA* 2001;286:1740–1747.
4. Brock I, Munk ME, Kok-Jensen A, Andersen P. Performance of whole blood IFN-γ test for tuberculosis diagnosis based on PPD or the specific antigens ESAT-6 and CFP-10. *Int J Tuberc Lung Dis* 2001;5: 462–467.
5. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* 2000;356:1099–1104.
6. van Pinxteren LA, Ravn P, Agger EM, Pollock J, Andersen P. Diagnosis of tuberculosis based on the two specific antigens ESAT-6 and CFP10. *Clin Diagn Lab Immunol* 2000;7:155–160.
7. Arend SM, Andersen P, Van Meijgaarden KE, Skjot RL, Subronto YW, van Dissel JT, Ottenhoff TH. Detection of active tuberculosis infection by T cell responses to early-secreted antigenic target 6-kDa protein and culture filtrate protein 10. *J Infect Dis* 2000;181:1850–1854.
8. Munk ME, Arend SM, Brock I, Ottenhoff TH, Andersen P. Use of ESAT-6 and CFP-10 antigens for diagnosis of extrapulmonary tuberculosis. *J Infect Dis* 2001;183:175–176.
9. Lalvani A, Pathan AA, Durkan H, Wilkinson KA, Whelan A, Deeks JJ, Reece WH, Latif M, Pasvol G, Hill AV. Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. *Lancet* 2001;357:2017–2021.
10. Arend SM, Engelhard AC, Groot G, De Boer K, Andersen P, Ottenhoff TH, van Dissel JT. Tuberculin skin testing compared with T-cell responses to *Mycobacterium tuberculosis*–specific and nonspecific antigens for detection of latent infection in persons with recent tuberculosis contact. *Clin Diagn Lab Immunol* 2001;8:1089–1096.
11. Noshiro T, Satoh K, Sato H, Miura Y. [Tuberculin survey of university students and postgraduates in 1998]. *Kekkaku* 2000;75:363–368.
12. Mori T. Recent trends in tuberculosis, Japan. *Emerg Infect Dis* 2000;6: 566–568.
13. Bellete B, Coberly J, Barnes GL, Ko C, Chaisson RE, Comstock GW, Bishai WR. Evaluation of a whole-blood interferon-γ release assay for the detection of *Mycobacterium tuberculosis* infection in 2 study populations. *Clin Infect Dis* 2002;34:1449–1456.
14. Villarino ME, Burman W, Wang YC, Lundergan L, Catanzaro A, Bock N, Jones C, Nolan C. Comparable specificity of 2 commercial tuberculin reagents in persons at low risk for tuberculous infection. *JAMA* 1999; 281:169–171.
15. Duchin JS, Jereb JA, Nolan CM, Smith P, Onorato IM. Comparison of sensitivities to two commercially available tuberculin skin test reagents in persons with recent tuberculosis. *Clin Infect Dis* 1997;25:661–663.
16. Villarino ME, Brennan MJ, Nolan CM, Catanzaro A, Lundergan LL, Bock NN, Jones CL, Wang YC, Burman WJ. Comparison testing of current (PPD-S1) and proposed (PPD-S2) reference tuberculin standards. *Am J Respir Crit Care Med* 2000;161:1167–1171.
17. Rooney JJ Jr, Crocco JA, Kramer S, Lyons HA. Further observations on tuberculin reactions in active tuberculosis. *Am J Med* 1976;60:517–522.
18. Hirsch CS, Toossi Z, Othieno C, Johnson JL, Schwander SK, Robertson S, Wallis RS, Edmonds K, Okwera A, Mugerwa R, *et al.* Depressed T-cell interferon-γ responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. *J Infect Dis* 1999; 180:2069–2073.
19. Pathan AA, Wilkinson KA, Klenerman P, McShane H, Davidson RN, Pasvol G, Hill AV, Lalvani A. Direct *ex vivo* analysis of antigen-specific IFN-γ-secreting CD4 T cells in *Mycobacterium tuberculosis*–infected individuals: associations with clinical disease state and effect of treatment. *J Immunol* 2001;167:5217–5225.
20. von Reyn CF, Horsburgh CR, Olivier KN, Barnes PF, Waddell R, Warren C, Tvaroha S, Jaeger AS, Lein AD, Alexander LN, *et al.* Skin test reactions to *Mycobacterium tuberculosis*–purified protein derivative and *Mycobacterium avium* sensitin among health care workers and medical students in the United States. *Int J Tuberc Lung Dis* 2001;5: 1122–1128.
21. Swaminathan S, Gong J, Zhang M, Samten B, Hanna LE, Narayanan PR, Barnes PF. Cytokine production in children with tuberculous infection and disease. *Clin Infect Dis* 1999;28:1290–1293.

22. Shams H, Wizel B, Weis SE, Samten B, Barnes PF. Contribution of $CD8^+$ T cells to γ interferon production in human tuberculosis. *Infect Immun* 2001;69:3497–3501.
23. Vekemans J, Lienhardt C, Sillah JS, Wheeler JG, Lahai GP, Doherty MT, Corrah T, Andersen P, McAdam KP, Marchant A. Tuberculosis contacts but not patients have higher γ interferon responses to ESAT-6 than do community controls in the Gambia. *Infect Immun* 2001;69: 6554–6557.
24. Doherty TM, Demissie A, Olobo J, Wolday D, Britton S, Eguale T, Ravn P, Andersen P. Immune responses to the *Mycobacterium tuberculosis*–specific antigen ESAT-6 signal subclinical infection among contacts of tuberculosis patients. *J Clin Microbiol* 2002;40:704–706.
25. Arend SM, Van Meijgaarden KE, De Boer K, De Palou EC, van Soolingen D, Ottenhoff TH, van Dissel JT. Tuberculin skin testing and *in vitro* T cell responses to ESAT-6 and culture filtrate protein 10 after infection with *Mycobacterium marinum* or *M. kansasii*. *J Infect Dis* 2002;186:1797–1807.
26. Vordermeier HM, Whelan A, Cockle PJ, Farrant L, Palmer N, Hewinson RG. Use of synthetic peptides derived from the antigens ESAT-6 and CFP-10 for differential diagnosis of bovine tuberculosis in cattle. *Clin Diagn Lab Immunol* 2001;8:571–578.
27. Food and Drug Administration, Center for Devices and Radiological Health. QuantiFERON-TB-P010033 [letter]. URL: www.fda.gov/cdrh/pdf/P010033b.pdf (Accessed February 7, 2004.)

一般演題14

車載型らせんCTを用いた胸部検診における経過観察例のCT所見

潤間隆宏[1]　鈴木公典[2]　大平尚子[2]　佐藤由梨[2]
小野崎郁史[2]　長尾啓一[3]　猪狩英俊[4]　天野佳子[5]
渡辺哲[5]　滝口裕一[5]　栗山喬之[5]

[目的]車載型らせんCTを用いた胸部検診において、経過観察と判定された群と、肺癌・異型腺腫様過形成と確定診断された群との間でthin-section CT所見の比較検討をおこなった。
[対象と方法]一自治体住民849名を対象として、車載型らせんCTを用いた一次検診を行なった。精密検査が必要と判定された例にthin-section CTを施行し、確定診断が必要な例は精査医療機関受診を推奨した。
[結果]要精査100例中83名に、精査CTを撮像した。CTガイド下生検・胸腔鏡下肺生検・開胸肺生検などの診断的検査により肺癌5例、AAH1例が診断された。2年間の経過観察継続および終了例は18例であった。経過観察継続例で他部位の陰影出現と消失が1例に、陰影の増大が1例に認められた。肺癌・AAH6名6病変のC群と経過観察継続・終了群18名22病変につき、thin-section CT所見の比較検討を行い、辺縁不整・辺縁不鮮明・内部のすりガラス濃度・air bronchogram・静脈関与が、各群間において所見の比率に有意な差がみられた。
[結論]辺縁不整・辺縁不鮮明・すりガラス濃度・air bronchogram・静脈関与の所見が悪性病変を示唆する所見と考えられた。
キーワード：　車載型らせんCT、thin-section CT、肺癌、経過観察、胸部検診

はじめに

低線量CTを用いた胸部検診により、より小さなそしてより早期の肺癌を発見することが可能になり、肺癌死亡率の低下への寄与が期待されている。しかし、検出した肺野結節は、thin-section CT所見でも良悪性の鑑別が難しく経過観察を行う例も多い。今回我々は、車載型らせんCTを用いた胸部検診において精査CTで経過観察と判定された例と、肺癌・異型腺腫様過形成と確定診断された例のthin-section CT所見の比較検討をおこなった。

[1]千葉県立東金病院
(〒283-8588　千葉県東金市台方1229)
e-mail:uruma-t@umin.ac.jp
[2]ちば県民保健予防財団
[3]千葉大学総合安全衛生管理機構
[4]千葉大学医学部附属病院感染症管理治療部
[5]千葉大学医学研究院加齢呼吸器病態制御学

方法

2001年度に、千葉県内一自治体住民849名を対象として、ちば県民保健予防財団が、車載型らせんCTを用いた一次検診を行った。CT装置は、日立メディコ社製CT-W950SRを用い、撮像条件は、120 kV, 50mA, スライス幅10 mm, テーブル移動速度20 mm/sec, 2秒/回転であった。読影は、比較読影支援システムを用いてCRT上で用い、一症例につき2名の読影医が独立して読影を行った。2名の読影医のうち、いずれか1名以上が要精査とした症例について、合同判定を施行し、B判定(異常なし)C判定(異常所見を認めるが精査を必要としない)、D1判定(活動性肺結核を強く疑う)、

D2 判定(活動性非結核病変を強く疑う)、D3 判定(循環器疾患)、D4 判定(縦隔腫瘍、胸壁腫瘍など)、E1 判定(肺癌の疑いを否定できない)、E2 判定(肺癌を強く疑う)のいずれかに判定した。要精査例は、ちば県民保健予防財団結核予防センター受診を推奨した。同センターで精査CTとしてconventiolal CTおよびthin-section CT を撮像し、確定診断が必要な例は精検医療機関に紹介し、経過観察とされた症例は同センターにて継続受診し、24ヶ月間の経過観察の経過を検討した。

結果

判定結果はB判定418名、C判定319名、D1 判定3、D2判定1名、D3判定3名、D4判定5名、E1判定36名、E2判定64名であった。D1およびD2判定で結核予防センターを受診したのは3例で1例は肺結核の診断がなされた。E1およびE2判定とされた計100名に結核予防センター受診を推奨し、83名が同センターを受診、13名が他医療機関を受診し、精査未受診は4名であった。

結核予防センター受診者 83 名の精査判定結果は、異常なしが12名、肺癌以外の呼吸器疾患で精査の必要なしは35名、他医療機関を紹介し診断確定は9名、他医療機関紹介で診断未確定は5名、経過観察例は22名であった。他医療機関を紹介し診断確定した9名の内訳は肺癌5名、異型腺腫様過形成1名、炎症性病変2名、肺真菌症1名であった。肺癌5名、異型腺腫様過形成1名は、CTガイド下生検・胸腔鏡下肺生検・開胸肺生検により診断された。経過観察例22名は、2年間の経過観察中に、経過観察が患者都合により中断が4名、経過観察終了としたのが8名、経過観察を継続した例が10名であった。経過観察継続例での所見の変化がみられたのは、経過中他部位に結節影出現し縮小が見られた例と、胸膜直下の多角形の結節影で、経過中間質性肺炎の悪化と結節影の増大が見られた例であった。

経過観察終了および継続例の18名22病変のF群と肺癌・異型腺腫様過形成と診断された6名6病変のC群の2群に分類して、初診時におけるthin-section CT所見の比較を行った(表1)。各所見の有無による各群間の比率の差異について、Fisher's exact probability test を用いて検定を行った。辺縁不整・辺縁不鮮明・内部のすりガラス濃度・air bronchogram・静脈関与が、各群間において所見の比率に有意な差がみられた。形としては不整型および多角形を呈する陰影が経過観察群にのみ認められた。

考察

肺結節におけるCT所見として、辺縁不整・境界不鮮明やspiculationは、悪性の特徴とされ[1]、辺縁整や境界鮮明な結節は良性であることが多いとされているが、辺縁の性状だけでは良悪性の鑑別が困難である例も多い。

近年、スクリーニング検査や胸部検診で行われるCTで発見される小さな肺結節影の評価には、thin-section CTによる辺縁や内部構造の所見の解析により良悪性の鑑別診断が行われている。内部濃度の評価では、すりガラス濃度を呈する結節は、炎症の消退過程もしくは、腺癌・異型腺腫様過形成であることが多いとされている。結節の形や胸膜との関係では、松本らの検討では、1cm 以下で多角形や扁平な結節、胸膜に接した半円形の結節は良性の所見とされている[2]。

近年、肺野の小結節影を呈する肺内リンパ節が、微小肺癌との鑑別診断が難しい例もあることで注目されている。肺内リンパ節は、悪性腫瘍との鑑別上重要であるが、多くは胸膜から15mm以内の距離にあり、形は円形ないしは楕円形で分葉を示す場合があり気管分岐部より下部に存在し、大きさは15mm以下であるとされている[3]。また、松本らによれば、円形、多角形の結節から、短いspiculationとは異なる長い線状陰影を認める場合があると報告されている[2]。また、兵頭らは、結節影から連続する線状影を認める例を12例中11例に認め[4]、胸膜陥入像やnotch, spiculaを伴ったり、新たに出現を認めたり[5]、増大を認める例[6]もある。今回の検討では、多角形を呈するないしは胸膜に接する陰影はF群のみに5例認められ、肺内リンパ節を疑わせる所見であった。

確定診断が得られない場合は、2年間の胸部X線やCTによる経過観察で増大が見られないことを確認する必要がある。経過観察継続例で結節影の増大が認められた例は、胸膜直下の多角形と結節影から連続する線状影を示し、肺内リンパ節が疑われたが、経過中間質性肺炎の悪化に伴い結節影の増大が見られた。良性病変と考えられるF群とC群のthin-section CT所見の比較では、辺縁不整・辺縁不鮮明・内部のすりガラス濃度・air bronchogram・静脈関与が、C群において所見の比率が有意に高値であった。今回対象とした例が少ないため、今後、より多くの症例での検討が必要と考えられた。

まとめ

24ヶ月の経過観察では他部位の陰影出現と消失が1例に、陰影の増大が1例に認められた。F群とC群では、thin-section CT所見で，辺縁不整・辺縁不鮮明・内部のすりガラス濃度・air bronchogram・静脈関与の有無に差が見られ、これらは悪性病変を示唆する所見と考えられた。

本論文は平成14年度結核予防千葉基金医学研究助成による助成を受けた。

文　献

[1] Zwirewich CV, Vedal S, Miller RR et al: Solitary pulmonary nodule: high-resolution CT and radiologic-pathologic correlation. Radiology, 1991; 79: 469-476
[2] 松本常男:肺結節の存在診断と鑑別診断について. 肺癌 2002; 42: 686-697
[3] Matsuki M, Noma S, Kuroda Y, et al: Thin-section CT features of intrapulmonary lymph nodes. Journal of Computer Assisted Tomography. 2001; 25: 753-756
[4] 兵頭剛、河野良寛、芝本健太郎、他: 肺内リンパ節のHRCT所見12症例の検討. 臨床放射線 2002; 47: 135-139
[5] 常塚宣男、斎藤　裕、増田信二、他: び慢性汎細気管支炎経過観察中に画像上出現した胸膜下肺内リンパ節の1症例. 日胸 1994; 53: 278-283
[6] 花岡孝臣、西村秀紀、高砂敬一郎、他:胸腔鏡下肺生検で判明した胸膜下肺内リンパ節の2例. 日本臨床外科学会雑誌 2000; 61: 2936-2939

CT findings of follow-up case in chest screening with mobile CT unit

Takahiro Uruma[*1], Kiminori Suzuki[*2], Naoko Odaira[*2], Yuri Sato[*2], Ikushi Onozaki[*2], Keiichi Nagao[*3], Hidetoshi Igari[*4], Yoshiko Amano[*5], Satoshi Watanabe[*5], Yuichi Takiguchi[*5], Takayuki Kuriyama[*5]

Chiba prefectural Togane Hospital[*1],
Chiba Foundation for Health Promotion and Disease Prevention[*2],
Safety and health organization, Chiba University[*3],
Department of Control and Treatment of Infectious Diseases, Chiba University Hospital[*4],
Department of respirology, graduate school of medicine, Chiba University[*5]

Purpose

In thoracic screening with mobile CT unit, thin-section CT findings were compared between lesion judged to be follow-up and lesion diagnosed as lung cancer or adenomatous hyperplasia.

Subjects and methods

For 849 people who lived in the local government, chest screening for lung cancer with mobile CT unit was performed. A participant recommended detailed examination underwent thin-section CT, and the participant recommended further examination underwent diagnostic examination.

Results

Detailed examination was recommended for 100 people, and 83 people underwent thin-section CT. Five lung cancer and one atypical adenomatous hyperplasia (AAH) were diagnosed by CT-guided biopsy, open lung biopsy or video-assisted thoracic surgery. 18 people underwent follow-up for less than 2 years. During follow-up, lesion appeared in other locus and reduced in one case, and lesion enlarged in another case. Between group C (6 lesion of six case in lung cancer and AAH) and group F (22 lesion in 18 follow-up case), there was a significant difference in the ratio of five diagnostic CT findings (ill-defined, irregular, ground-grass opacity, air bronchogram and venous involvement).

Conclusion

CT findings of ill-defined, irregular, ground-grass opacity, air bronchogram and venous involvement suggested malignant lesion.

Key words: Mobile CT unit, Thin-section CT, Lung cancer, Follow-up, Chest screening

表1 F群とC群でのthin-section CT所見の比較

		F群	C群	
症例数		18	6	
陰影の数		22	6	
長径平均	mm	8	13	
長径範囲	mm	5-32	6-22	
辺縁性状	不整	9	6	*
	不鮮明	5	5	*
	分葉	0	1	
	spiculation	2	3	
内部構造	すりガラス濃度	4	4	*
	不均一	9	4	
	空洞	0	0	
	air bronchogram	1	4	**
	石灰化	1	0	
既存構造との関係	血管気管支の集束	5	3	
	静脈関与	3	4	*
	胸膜陥入	2	2	
	胸膜陥凹	0	1	
	胸膜肥厚	1	0	
	satellite lesion	3	0	
形	円形, 楕円形	11	6	
	不整型	6	0	
	多角形	5	0	
胸膜との関係	胸膜に接する	6	0	
	胸膜直下	4	3	

*: $p<0.05$, **: $p<0.01$

非小細胞肺癌の組織型からみた喫煙と呼吸機能障害の関連

木村　弘[1]・滝口裕一[2]・杉本尚昭[2]・福岡和也[1]・
宮澤　裕[2]・尾辻瑞人[3]・長尾啓一[4]・藤澤武彦[3]・
栗山喬之[2]

[1]奈良県立医科大学内科学第二講座；
千葉大学大学院医学研究院 [2]加齢呼吸器病態制御学，[3]胸部外科学；
[4]千葉大学保健管理センター

Japanese Journal of Lung Cancer

肺　癌　第44巻 第4号 2004年8月

ORIGINAL ARTICLE

非小細胞肺癌の組織型からみた喫煙と呼吸機能障害の関連

木村　弘[1]・滝口裕一[2]・杉本尚昭[2]・福岡和也[1]・
宮澤　裕[2]・尾辻瑞人[3]・長尾啓一[4]・藤澤武彦[3]・
栗山喬之[2]

要旨――目的・方法．肺扁平上皮癌（Sq）や肺腺癌（Ad）に対する化学療法や放射線療法に関して，呼吸機能障害の程度により治療内容を考慮すべきであると考えられる．しかし，現状では，SqとAdは非小細胞肺癌として一括して扱われており，両組織型間における肺傷害の差異や喫煙がこれらの呼吸機能障害に及ぼす影響については明らかにされていない．1995年から1999年までに千葉大学附属病院に入院した非小細胞肺癌患者について，気管支鏡にて中枢（区域気管支入口部まで）の病変の有（中枢型），無（末梢型）を評価し得た352例（Sq：136例，Ad：216例）のうち，重喫煙群（喫煙指数≧800：159例）と軽喫煙群（喫煙指数≦400：148例）の計307症例（Sq：117例，Ad：190例）を対象として，喫煙指数，病変部位，診断時の呼吸機能検査値の関連をretrospectiveに解析した．結果．対象症例全体の解析では，SqはAdと比べて年齢と喫煙指数はともに高く，拘束性換気障害，閉塞性換気障害，肺拡散障害，$AaDO_2$の開大がより顕著であった．一方，対象を末梢型肺癌に限った検討では，全症例についての解析とほぼ同様の傾向を認めたが，SqではAdに比べて肺拡散障害がより顕著であった．さらに，末梢型肺癌を喫煙指数にて層別化し検討した結果，呼吸機能検査値は，両組織型とも軽喫煙群に比べて重喫煙群で，より低下していたが，Sqでは$\dot{V}_{25}$/Htと肺拡散能は両喫煙指数群間で差異を認めず，軽喫煙群でも低下していた．結論．SqではAdに比べて呼吸機能障害が強く認められた．末梢型肺癌においては，両組織型ともに喫煙が呼吸機能障害に影響を及ぼしていたが，SqではAdに比べて軽喫煙群でも末梢気道障害や肺拡散障害を呈することが多いことが明らかにされた．（肺癌．2004;44:219-224）

索引用語――非小細胞肺癌，喫煙，肺傷害，呼吸機能障害

Disturbance of Respiratory Function Depends on Smoking History and Histological Type in Non-small Cell Lung Cancer

Hiroshi Kimura[1]; Yuichi Takiguchi[2]; Naoaki Sugimoto[2]; Kazuya Fukuoka[1]; Hiroshi Miyazawa[2]; Mizuto Otsuji[3]; Keiichi Nagao[4]; Takehiko Fujisawa[3]; Takayuki Kuriyama[2]

ABSTRACT ―― ***Objective.*** When chemotherapy is conducted for the treatment of non-small cell lung cancer (NSCLC), we must pay attention to the degree of impairment of pulmonary function. So far, it is not clear whether the smoking affects pulmonary function in a different manner according to the histological types of lung cancer; squamous cell carcinoma and adenocarcinoma of the lung. In order to clarify these issues, we investigated the relation of smoking index (SI), location of cancerous lesions, and pulmonary function in patients with NSCLC. ***Study design.*** A total of 307 cases (squamous cell carcinoma; 117, adenocarcinoma; 190), with bronchial lesions located at sites proximal to the ori-

[1]奈良県立医科大学内科学第二講座；千葉大学大学院医学研究院 [2]加齢呼吸器病態制御学，[3]胸部外科学；[4]千葉大学保健管理センター．

別刷請求先：木村　弘，奈良県立医科大学内科学第二講座，〒634-8522　奈良県橿原市四条町840（e-mail: kimura@nmu-gw.naramed-u.ac.jp）．

[1]Second Department of Internal Medicine, Nara Medical University, Japan; Department of [2]Respirology, [3]Thoracic Surgery, Graduate School of Medicine, Chiba University, Japan; [4]Chiba University Health Sciences Center, Japan.

Reprints: Hiroshi Kimura, Second Department of Internal Medicine, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan (e-mail: kimura@nmu-gw.naramed-u.ac.jp).

Received May 17, 2004; accepted July 15, 2004.

fice of segmental bronchi (central lesions), or beyond (peripheral lesions) using fiberoptic bronchoscopy, was divided into two groups according to SI. There were 159 cases with an SI of more than 800 (high SI), and 148 less than 400 (low SI). ***Results.*** Age and SI were higher in squamous cell carcinoma than in adenocarcinoma, and restrictive and obstructive disturbances and a decrease in DLco and widened alveolar-arterial oxygen difference ($AaDO_2$) were more prominent in squamous cell carcinoma than in adenocarcinoma. Similar results were obtained from analyses in the patients with peripheral lesions. In particular, disturbance of diffusion capacity was more prominent in squamous cell carcinoma than in adenocarcinoma. Moreover, when the subjects were limited to the patients with peripheral lesions, pulmonary functions in the high SI group tended to be more markedly disturbed than in the low SI group. In squamous cell carcinoma, however, $\dot{V}_{25}$/Ht and DLco did not show a significant difference between high SI and low SI groups with deteriorations of these parameters being observed even in patients with low SI. ***Conclusion.*** It is concluded that the disturbance of respiratory function is more prominent in squamous cell carcinoma than in adenocarcinoma. Smoking affects the disturbance of respiratory functions in NSCLC with peripheral lesions. Squamous cell carcinoma shows greater impairment of the peripheral airways and diffusion capacity than adenocarcinoma, even in patients with an SI of less than 400. (*JJLC.* 2004;44:219-224)

KEY WORDS —— Non-small cell lung cancer, Smoking, Lung injury, Disturbance of respiratory function

はじめに

近年，非小細胞肺癌のガイドラインが相次いで発表されてきた．わが国でも米国 (American Society of Clinical Oncology: ASCO)[1] でも，肺腺癌 (adenocarcinoma: Ad) と肺扁平上皮癌 (squamous cell carcinoma: Sq) は治療効果において概ね差異はないとしているものの，両組織型間における有害事象の差異に関しては，ほとんど触れられていない．

このような現状のなかで，わが国において世界に先駆けて臨床の場に登場したゲフィチニブ (イレッサ®) の肺傷害発症[2,3] の危険因子は，多変量解析の結果から，女性より男性で，非喫煙者より喫煙者で，また，Ad より Sq であることが明らかにされた．[4,5]

非小細胞肺癌の化学療法や放射線療法においては，呼吸器合併症や呼吸機能障害の程度により治療内容を考慮すべきであると考えられてはきたものの，呼吸機能障害における，喫煙と組織型の関連に関しては十分には理解されているとは言い難い．したがって，呼吸機能障害に及ぼす喫煙の影響が肺癌組織型によって異なるか否かについてを明確にすることは，有害事象の観点から臨床の場で意義深いと考えられる．そのような背景のなかで，Sq と Ad における呼吸機能障害の差異と喫煙の影響について，特に，Sq で Ad より呼吸機能障害が顕著なのは，1) 喫煙自体の影響なのか，2) 肺組織型の特徴なのかについて検討した．

対象と方法

1995 年から 1999 年までに千葉大学医学部附属病院呼吸器内科および同呼吸器外科に入院した非小細胞肺癌患者について，気管支鏡にて中枢 (区域気管支入口部まで) の病変の有 (中枢型)，無 (末梢型) を評価し得た 352 例 (Sq：136 例，Ad：216 例) のうち，重喫煙群 (喫煙指数≧800：159 例) と軽喫煙群 (喫煙指数≦400：148 例) の計 307 症例 (Sq：117 例，Ad：190 例) を検討対象とした (Table 1)．対象症例の年齢分布は 31～85 歳で，平均年齢は 63.5 歳であった．性別は，男性 203 例，女性 104 例であった．これらの症例について，診断時の呼吸機能諸検査値と喫煙指数，気管支鏡における中枢型・末梢型別の病変部位，病理組織型との関連について retrospective に解析を行った．なお，肺拡散能 (DLco) に関しては，160 例 (Sq：67 例，Ad：93 例) において施行した．有意差検定は Student's t test および χ^2 検定を用い，$p<0.05$ を有意差ありとした．また，多変量解析には，SAS ver 8.2 を用いた．

結　果

Sq と Ad の平均年齢は各々 66.1 歳，61.9 歳で Sq で有意に高齢であり，かつ，男性の割合は各々 92.6%，54.6% で Sq で有意に高かった．また，Sq は Ad と比較して喫煙指数が有意に高く，さらに，喫煙指数≧800 (重喫煙群) の割合は各々 60.9%，32.7% と Sq でより高値を示した．診断時の呼吸機能検査では，Sq では Ad に比べて，%FVC，FEV_1%，%PEF (% peak expiratory flow)，$\dot{V}_{25}$/Ht，% ($DLco/V_A$) は低値を示し，$AaDO_2$ の開大が顕著であった (Table 1)．呼吸機能の各項目において年齢，性別，組織型，喫煙指数，中枢型/末梢型を説明因子として多変量解析を行った．説明因子間の相互作用を考慮した

Table 1. Statistical Differences in Ages, Smoking Indices and Pulmonary Function Data Between Squamous Cell Carcinoma (Sq) and Adenocarcinoma (Ad) of the Lung

Location Histology	No. of cases	Age	Sex (M : F)	SI	%FVC	FEV1%	%PEF	$\dot{V}_{25}$/Ht	% (DLco/VA)	PaO_2	$AaDO_2$
Total											
Sq	117	66.1 (9.2)	108 : 9	1211 (747)	87.1 (20.8)	72.8 (11.7)	65.2 (24.6)	0.41 (0.27)	65.0 (25.3)	83.5 (10.9)	18.8 (10.7)
Ad	190	61.9 (10.6) ‡	95 : 95 ‡	531 (665) ‡	93.2 (19.1) †	79.7 (11.3) †	73.0 (26.5) *	0.50 (0.25) †	76.5 (25.7) †	85.4 (11.8)	15.4 (10.7) †
Peripheral type											
Sq	62	67.8 (8.1)	57 : 5	1156 (726)	88.7 (21.4)	74.1 (12.7)	68.8 (25.9)	0.44 (0.29)	62.7 (22.5)	84.3 (12.1)	17.5 (10.9)
Ad	153	62.4 (10.3) ‡	74 : 79 ‡	532 (1052) ‡	94.6 (19.2)	80.3 (11.1)	73.5 (26.7)	0.51 (0.26)	74.8 (24.1) †	85.8 (12.0)	15.0 (10.8)
Central type											
Sq	55	64.1 (9.7)	51 : 4	1272 (823)	85.2 (20.1)	71.4 (11.2)	61.1 (20.3)	0.37 (0.24)	68.5 (17.1)	82.7 (10.7)	20.2 (11.3)
Ad	37	60.1 (12.0)	21 : 16 ‡	527 (668) ‡	87.5 (16.3)	77.0 (10.9)	71.3 (23.9) *	0.46 (0.24)	83.3 (34.1)	84.0 (11.6)	17.1 (11.8)

Sq: squamous cell carcinoma; Ad: adenocarcinoma; M: male; F: female; SI: smoking index.
Data represent means and SD with in parentheses, * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$ between Sq and Ad in each type.

場合，FEV1%，%PEF，$\dot{V}_{25}$/Ht，% (DLco/VA) の項目において組織型が有意な因子として選択され，これらの呼吸機能に影響を及ぼす組織型の重要性が示された．

さらに，対象を末梢型に限った検討では，Sq では Ad より年齢と喫煙指数はともに高く，% (DLco/VA) は Sq で有意に低値を示した．一方，中枢型においては，%PEF が Sq で有意に低下していた (Table 1)．

末梢型に関して，各組織型間での重喫煙群と軽喫煙群との比較では，両組織型とも重喫煙群では%FVC，FEV1%，PaO_2 は低下しており，$AaDO_2$ の開大も増大していた．一方，Ad でのみ，% (DLco/VA) と $\dot{V}_{25}$/Ht は軽喫煙群に比べて重喫煙群で低下を認めたが，Sq では両喫煙群間で差異は認めなかった．さらに，軽喫煙群においては，FEV1% と% (DLco/VA) は，Sq にて Ad より有意に低下していた (Figure 1, 2)．また，重喫煙群かつ末梢型における両組織型間の検討では，年齢以外の呼吸機能検査値には差異を認めなかった (Figure 1, 2)．

考　察

本研究においては，区域気管支入口部より中枢側に可視病変を伴う中枢型では，peak flow rate の有意な低下がみられた．また，中枢気道病変の進行によって，無気肺による肺活量の低下等も早期に伴いやすいことより，末梢型に着目して検討を行った．

今回の検討結果は以下のように要約し得た．1) Sq は Ad と比べて，年齢と喫煙指数がともに高く，拘束性換気障害や閉塞性換気障害や肺拡散障害の程度，さらに $AaDO_2$ の開大がより顕著であった．2) 末梢型肺癌において，喫煙指数にて層別化した結果，両組織型とも重喫煙群では呼吸機能検査値がより低下する傾向を認めたが，$\dot{V}_{25}$/Ht と肺拡散能は，Ad においてのみ重喫煙群が軽喫煙群に比べて低下していた．一方，Sq では両喫煙群間で差異は認めず，軽喫煙群であっても低下していることが示唆された．実際に，日本人臨床肺機能検査指標標準値[6]との比較検討では，$\dot{V}_{25}$/Ht は両組織型とも，軽喫煙群においても国民標準値より低下していた (0.63±0.16 (年齢補正国民標準値) vs. 0.52±0.24 l/sec/m (Sq：本検討低喫煙群)；$p<0.01$，0.74±0.18 (年齢補正国民標準値) vs. 0.55±0.28 l/sec/m (Ad：本検討低喫煙群)；$p<0.001$)．

本検討では，Sq では Ad に比べて呼吸機能障害が強いが，これには喫煙指数が重要な因子となりうることが改めて確認された．つまり，非小細胞肺癌に対する化学療法等における肺傷害リスクを軽減するためには，組織型にかかわらず，まず喫煙の影響を十分考慮すべきであると考えられた．喫煙と肺癌発症との関連では，これまで男性喫煙者には Sq が多いとされてきたが，最近では 50 歳代および 60 歳代の男性喫煙者において，Ad の比率が

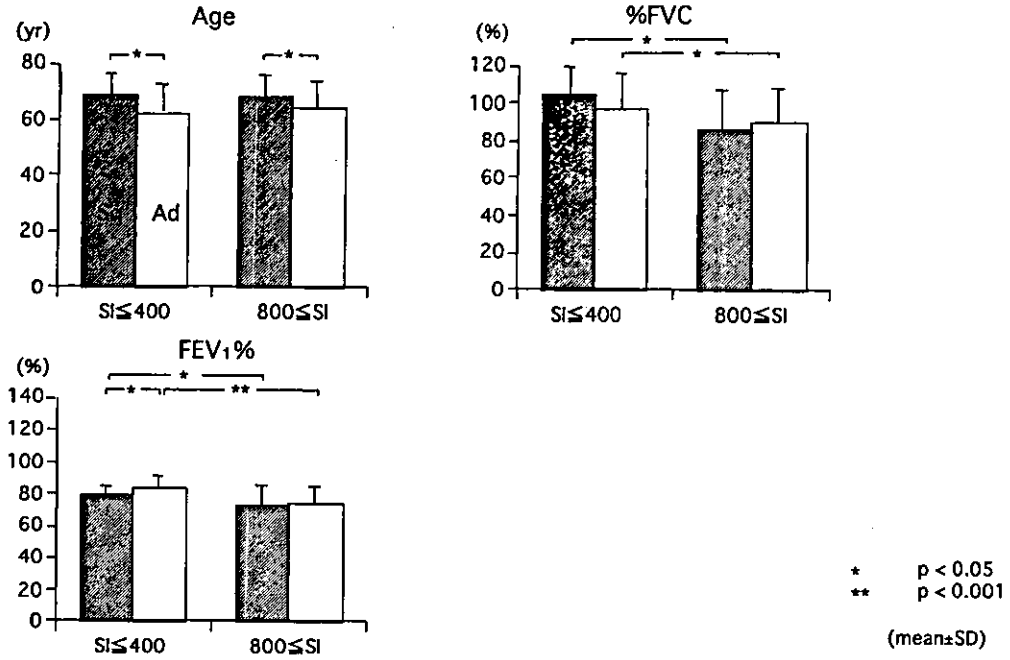

Figure 1. Comparison of age and pulmonary functions according to histological types and smoking indices in lung cancer patients with peripheral lesions.

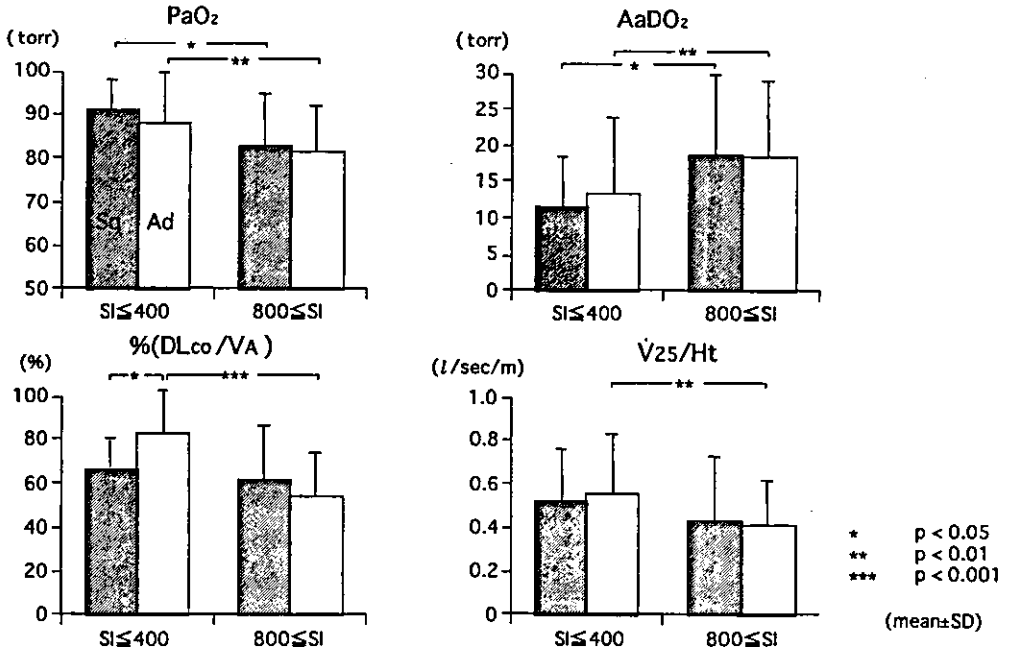

Figure 2. Comparison of arterial blood gas analyses and pulmonary functions according to histological types and smoking indices in lung cancer patients with peripheral lesions.

増加していることが報告されている.[7] これには，フィルター付きたばこの普及により，中枢気管支に沈着しやすかった大きな粒子が除去されるようになったこと，煙を深く吸い込みやすくなったこと，Adの発癌物質であるニトロサミンのたばこ中の濃度が上昇していることなどが考えられている.[7,8]

さらに，末梢型肺癌に限定して喫煙指数によって層別化した検討からは，喫煙指数が800以上の重喫煙群においてはSqもAdもほぼ同様の呼吸機能障害を認めたものの，喫煙指数が400以下の軽喫煙群においては，Sqでは末梢気道の閉塞性障害の指標と考えられる$\dot{V}_{25}$/Htの低下および肺拡散能の有意な低下を認めることが新たに明らかとなった．このように，Sqでは軽喫煙群においても，末梢気道障害や肺拡散障害をAdより呈しやすいことから，Sqにおいては，肺癌による二次的な呼吸機能への影響が早期から生じやすい可能性も考えられる．今後，呼吸機能検査のみならず，HRCTなどによる画像的評価も加味したCOPDの合併頻度を，各組織型および喫煙指数に応じて検討していく必要性があると考えられた．さらに，Sqにおいては発癌と肺傷害とは共通の危険因子を有する可能性もある．一般に，喫煙によるたばこ煙は線毛障害物質も含有するため，気道のクリアランス機能が障害されることで，発癌物質の肺内での貯留や沈着が進み，間接的にも発癌性を増強する可能性も指摘されている.[9]Sqにおいて，なぜ軽喫煙群の段階から末梢気道障害や肺拡散障害を呈することが多いかについては，気道クリアランス機能の障害をも踏まえ，さらなる検討が必要と考えられた．

近年の非小細胞肺癌のガイドラインでは，わが国においてもASCOからの報告においても，ともにAdとSqの治療効果に概ね差異はないとしているものの，両組織型における有害事象の差異に関しては，ほとんど触れられていない．わが国におけるゲフィチニブの肺傷害では，AdよりSqで，非喫煙者より喫煙者で発症リスクが高いことが報告されてきたが，本研究から得られた結果，つまり，喫煙量のみならず，Sqで喫煙量が少ない時期でも肺傷害が加わっている可能性が示唆されたことは，ゲフィチニブの肺傷害はSqでより顕著に起こりやすいことと共通の背景を有している可能性が考えられた．

非小細胞肺癌の呼吸機能に関する検討は数多くなされてきたが，治療との関連では，術後の呼吸器合併症発症の危険因子として，術前化学療法による肺拡散障害が重要であるとの報告が注目されている.[10,11] 一般に，SqやAdの化学療法や放射線療法においては，呼吸器合併症の程度により治療内容を考慮すべきことは言うまでもない．また，呼吸機能障害の程度によっても治療方針を考慮すべきであるが，この際，喫煙指数のみならず，Sqでは肺組織型自体の特徴として，喫煙指数の少ない時期より末梢気道障害や肺拡散障害を惹起しやすいことも考慮して治療にあたるべきであると考えられる．

近年の非小細胞肺癌の化学療法においては，比較的新しい抗がん剤がラインナップに加わったが,[12] その多くは肺毒性を有することが報告されている．特に，局所進展型に対しては，これら化学療法に積極的に胸部放射線照射が併用され,[13-16] しかも同時併用により，一層の効果が得られるとする立場が優勢である.[17] このような状況でより多くの患者が肺毒性の強い治療を受けることにより，組織型の違いによる肺傷害の差異が将来的に問題になることも否定できず，ゲフィチニブに限らず，肺癌治療における肺傷害の有害事象を論じる際には組織型ごとの検討も必要になると考えられた．

まとめ

以上，1) SqではAdに比べて呼吸機能障害は強いが，これには喫煙指数が重要な因子となりうる．このことより，非小細胞肺癌に対する化学療法等における肺傷害リスクを軽減するには，組織型にかかわらず喫煙の影響を十分考慮すべきである．2) Sqにおいては，軽喫煙群でも末梢気道障害や肺拡散障害を呈することが多いことより，癌による二次的な呼吸機能への影響とともに，発癌と肺傷害とが共通の危険因子を有する可能性も考えられ，さらなる検討を要する課題と考えられた．

REFERENCES

1. Pfister DG, Johnson DH, Azzoli CG, et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. *J Clin Oncol*. 2004;22:330-353.
2. Inoue A, Saijo Y, Maemondo M, et al. Severe acute interstitial pneumonia and gefitinib. *Lancet*. 2003;361:137-139.
3. Okamoto I, Fujii K, Matsumoto M, et al. Diffuse alveolar damage after ZD1839 therapy in a patient with non-small cell lung cancer. *Lung Cancer*. 2003;40:339-342.
4. ゲフィチニブ（イレッサ®錠250）の急性肺障害・間質性肺炎(ILD)に関する専門家委員会最終報告(2003. 3. 26)．アストラゼネカ．
5. Takeda T, Yamamoto N, on behalf of West Japan Thoracic Oncology Group (WJTOG). An epidemiological survey for interstitial lung disease induced by gefitinib in patients with advanced non-small cell lung cancer. *Abstr of the 10th World Conference on Lung Cancer*. 2003;S250.
6. 日本胸部疾患学会肺生理専門委員会．日本人臨床肺機能検査指標基準値．全国14施設における健常者肺機能検査成績の集計．日本胸部疾患学会雑誌．1993;31 (3)．
7. 岡本佳裕，山崎浩一，原田敏之，他．1980年代から1990年代への原発性肺癌の組織型の変遷とその背景因子の検討．肺癌．2000;40:609-614.
8. Thun MJ, Lally CA, Flannery JT, et al. Cigarette smoking

and changes in the histopathology of lung cancer. *J Natl Cancer Inst*. 1997;89:1580-1586.
9. 厚生省，編．喫煙と健康—喫煙と健康問題に関する報告書．第2版．東京：保健同人社；1993:1-334.
10. Fujiu K, Kanno R, Suzuki H, et al. Preoperative pulmonary function as a predictor of respiratory complications and mortality in patients undergoing lung cancer resection. *Fukushima J Med Sci*. 2003;49:117-127.
11. Leo F, Solli P, Spaggiari L, et al. Respiratory function changes after chemotherapy: an additional risk for postoperative respiratory complications? *Ann Thorac Surg*. 2004;77:260-265.
12. Socinski MA, Morris DE, Masters GA, et al. Chemotherapeutic management of stage IV non-small cell lung cancer. *Chest*. 2003;123:226S-243S.
13. Schaake-Koning C, van den Bogaert W, Dalesio O, et al. Effects of concomitant cisplatin and radiotherapy on inoperable non-small-cell lung cancer. *N Engl J Med*. 1992; 326:524-530.
14. Le Chevalier T, Arriagada R, Quoix E, et al. Radiotherapy alone versus combined chemotherapy and radiotherapy in unresectable non-small cell lung carcinoma. *Lung Cancer*. 1994;10:S239-244.
15. Dillman RO, Herndon J, Seagren S, et al. Improved survival in stage III non-small-cell lung cancer: seven-year follow-up of cancer and leukemia group B (CALGB) 8433 trial. *J Natl Cancer Inst*. 1996;88:1210-1215.
16. Jeremic B, Shibamoto Y, Acimovic L, et al. Hyperfractionated radiation therapy with or without concurrent low-dose daily carboplatin/etoposide for stage III non-small-cell lung cancer: a randomized study. *J Clin Oncol*. 1996; 14:1065-1070.
17. Furuse K, Fukuoka M, Kawahara M, et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small-cell lung cancer. *J Clin Oncol*. 1999;17:2692-2699.