with picking and/or packing, and 12 workers were involved with a variety of responsibilities related to growth/maintenance. Nine workers complained of a dry cough, shortness of breath and/or low grade fever. Three of the 9 workers reported acute symptoms consistent with HP. On the nasal cavity culture, *P. citrinum* was isolated from 54.2% of the workers, while Enoki was isolated from only 8% of the workers (Table I).

In blood samples, KL-6 levels ranged from 152 to 6,683 U/ml (mean, 516.3 U/ml), and were positive in seven workers (14.6%). SP-A levels ranged from 9.7 to 108 ng/ml (mean, 33.7 ng/ml), and were positive in five workers (10.4%). SP-D levels ranged from 17.2 to 437 ng/ml (mean, 68.4 ng/ml), and were positive in five workers (10.4%) (Table II).

LST values exceeding 200% are considered to be positive according to SRL. However, LST values to *P. citrinum* of normal controls ranged from 198% to 360% (mean, 284.4%) in this study. The cut-off level was finally decided to be 400% based on the data of normal controls. In the same way, LST values to Enoki and the culture medium were examined. LST values to Enoki and the culture medium of normal controls ranged from 267% to 380% (mean, 330%) and from 185% to 346% (mean, 281%), respectively. The cut-off levels in Enoki and the culture medium were finally decided to be both 400%. LST revealed a high positive rate for each antigen. LST values to *P. citrinum*, Enoki, and the culture medium were positive in 22 (45.8%), 26 (54.2%), and 26 (54.2%) workers, respectively. Precipitating lines were observed to *P. citrinum* in five workers (10.4%) in DIT. There were no precipitating lines observed to Enoki or culture medium in any of the workers. Normal controls had no precipitins to either *P. citrinum*, Enoki or the culture medium (Table II).

TABLE I. Characteristics of the 48 Enoki Workers

	Total	Male	Female
Subjects	48	23	25
Age (years old)	53.1 ± 10.6	53.3 ± 10.4	52.9 ± 11.1
History of allergy	6 (12.5%)	2 (8.7%)	4 (16%)
Smoking history			
Current-smoker	13 (27.1%)	12 (52%)	1 (4%)
Ex-smoker	7 (14.6%)	4 (17.4%)	3 (12%)
Never-smoked	28 (58.3%)	7 (30%)	21 (84%)
Duration of working (years)	18.4 ± 9.6	19.3 ± 8.4	17.5 ± 10.7
Symptoms	9 (18.8%)		
Dry cough and sputum	6 (12.5%)	1 (4.3%)	5 (20%)
Shortness of breath	4 (8.3%)	2 (8.7%)	2 (8%)
Fever	1 (2.1%)	0 (0%)	1 (4%)
Culture of nasal cavity	26 (54.2%)[a]	13 (56.5%)	13 (52%)

[a]Percentage of workers from whom *Penicillium citrinum* was isolated.

Further Examinations

Nine workers met the criteria for further examinations. The subjects consisted of 3 males and 6 females, and 6 workers were working as pickers and/or packers, and 3 workers were engaged in everything. Six of the 9 workers demonstrated respiratory symptoms such as dry cough, shortness of breath, and low grade fever. Shortness of breath was very severe in cases 1, 8, and 9, and their Fletcher-Hugh-Jones levels ranged around III–IV. In cases 1, 6, 8, and 9, respiratory symptoms resolved within several days of being away from the work environment, and then reappeared gradually after they returned to work again. CXR demonstrated diffuse ground glass opacity (GGO) in both lung fields in cases 1, 8, and 9, and their chest CT images showed centrilobular small nodule as well as GGO. In case 6, there was no abnormal shadow on the CXR, but the chest CT demonstrated bilateral diffuse GGO (Table III).

Total cell counts in the BALF ranged from 11.9×10^4 to 309×10^4 (mean, 67.3×10^4), and the percentage of lymphocytes ranged from 13.2% to 91.5% (mean, 57.2%). The CD4/CD8 ratio of the lymphocyte surface markers ranged from 0.3 to 2.5 (mean, 1.26). Specimens obtained by TBLB showed lymphocyte infiltrated alveolitis in cases 1, 6, 8, and 9, and a Masson body in case 9. There was no epitheloid noncaseating granuloma in specimens obtained from any of the workers examined. In the BALF obtained from case 6, *Penicillium* spp. was cultured on PDA but it was not possible to identify the species (Table IV).

LST in BALF was performed to *P. citrinum*, Enoki and the culture medium. The LST values in BALF to *P. citrinum* were positive in cases 1, 5, 6, and 9 though most cases had positive reactions to *P. citrinum* on LST in sera. In case 9, the LST value to *P. citrinum* was negative in serum but positive in BALF. Positive serum precipitin findings to *P. citrinum* were shown in cases 1, 6, 8, and 9 (Table V). Although no inhalation challenge to *P. citrinum* was performed in the 9 selected workers, cases 1, 6, 8, and 9 were finally diagnosed as having HP caused by *P. citrinum* based on the diagnostic criteria of HP.

Biomarkers were compared among workers (Table VI). KL-6 and SP-D in sera were significantly higher in the HP group ($n = 5$) than those in the non-HP group ($n = 44$, $P = 0.0004$, $P = 0.0037$, respectively). There was no significant difference in SP-A between the non-HP group and the HP group. The LST values in sera to *P. citrinum* were significantly higher in the HP group than those in the non-HP group ($P = 0.011$). The LST values in BALF to *P. citrinum* were also examined in the 9 selected workers and the initial HP patient (non-HP group, $n = 5$ vs. HP group, $n = 5$). The LST values were significantly higher in the HP group ($2,772 \pm 2,124.2$) than those in the non-HP group (203 ± 213.4, $P = 0.027$). The area under the curve (AUC) in a receiver operating characteristic (ROC) analysis was the

TABLE II. Laboratory Data of the Blood Samples From the 48 Enoki Workers

	Average		Positive (%)
KL-6 (U/ml) (≥450 U/ml)	516.3 ± 1,096.4		7/48 (14.6)
SP-A (ng/ml) (≥43.8 ng/ml)	33.7 ± 16.5		5/48 (10.4)
SP-D (ng/ml) (≥110 ng/ml)	68.4 ± 82.0		5/48 (10.4)
	LST		
	LST value (%)	Positive (%)[a]	Double immunodiffusion test (%)
Penicillium citrinum	497.8 ± 584.9	22/48 (45.8)	5/48 (10.4)
Enoki	652.8 ± 658.5	26/48 (54.2)	0/48 (0)
Culture medium	554.9 ± 421.0	26/48 (54.2)	0/48 (0)

KL-6, Krebs von der Lungen-6; SP-A, surfactant protein-A; SP-D; surfactant protein-D; LST, lymphocyte stimulation test.
[a]LST value ≥400% is considered to be positive on each antigen.

best of 0.99 for KL-6 when the cut-off level was 450 U/ml. The AUC for SP-D was as good as 0.90 when the cut-off level was 110 ng/ml. The AUC in LST was 0.87 when the cut-off level was 400%. The sensitivity and specificity of KL-6 showed 100% and 93.2%, respectively. The sensitivity and specificity of DIT showed 100% and 97.5%, respectively. In the LST, the sensitivity was as good as 80%, but the specificity was as low as 56.8%.

DISCUSSION

In this study, workers were selected showing KL-6 ≥450 U/ml, SP-A ≥43.8 ng/ml or SP-D ≥110 ng/ml with a positive response to *P. citrinum* on LST or DIT as potential HP patients. Previous reports have investigated the criteria by which HP patients should be diagnosed. Tsushima et al. [2005] reported that serum KL-6 and SP-D may be related to the resolution of HP in addition to the LST values and chest CT. Kohno et al. [1989] and Kobayashi and Kitamura [1995] reported that the serum KL-6 is a good marker for disease activity in interstitial pneumonitis such as HP. SP-A and SP-D are also known to be good markers indicating disease activity of HP [Abe and Takahashi, 2000; Tanaka et al., 2000]. LST is sometimes used to screen patients and to find the causative antigen in some types of HP such as MWL [Tsushima et al., 2001]. In this study, KL-6, SP-D, and LST values in sera were significantly higher in the HP group than those in the non-HP group.

An ROC analysis was conducted to investigate the usefulness of these biomarkers as predictors. The AUC was the best of 0.99 for KL-6 when the cut-off level was determined to be 450 U/ml, and it was thought that KL-6 was the most useful as a predictor of HP in this study and that the cut-off level was appropriate. The AUC in LST was 0.87 when the cut-off level was determined to be 400%. This result suggested that the cut-off level of 400% might be appropriate. However, the positive rate in LST to *P. citrinum* was very high (45.8%) (Table II), and LST had low

TABLE III. Further Examinations: 9 Selected Workers and the Initial Patient

Case no.	Age, year/sex	Smoking history	Duration of working, year	Job	Symptoms	CXR	CT
(A) non-HP (n = 5)							
2	51/F	Never	0.25	Picker	Dry cough	Normal	Normal
3	54/M	Never	22	All	SOB	Normal	Normal
4	62/F	Never	37	Packer	(−)	Normal	Normal
5	51/M	Ex	21	All	(−)	Normal	Normal
7	72/F	Never	40	Packer	(−)	Normal	Normal
(B) HP (n = 5)							
1	54/F	Never	20	Picker/packer	Dry cough, SOB	GGO	GGO, CLSN
6	43/F	Never	3	Picker/packer	Dry cough	Normal	GGO
8	72/F	Never	16	Packer	Fever, SOB	GGO	GGO, CLSN
9	37/M	Ex	17	All	SOB	GGO	GGO, CLSN
Patient	47/F	Never	21	Picker/packer	Dry cough, SOB	GGO	GGO, CLSN

SOB, shortness of breath; GGO, gland-glass opacity; CLSN, centrilobular small nodule.

TABLE IV. BALF and TBLB Findings: 9 Selected Workers and the Initial Patient

Case no.	Cell counts, 10^4 cells/ml	Lymphocytes (%)	CD4/CD8	TBLB	Culture *Penicillium* spp. of BALF
(A) non-HP (n = 5)					
2	37.0	24.2	0.6	Normal	–
3	39.5	68.3	0.6	Normal	–
4	36.2	13.2	2.5	Normal	–
5	38.8	25.6	0.7	Normal	–
7	25.4	64.5	2.4	Normal	–
(B) HP (n = 5)					
1	45.8	91.5	0.36	Alveolitis	–
6	309.0	81	0.3	Alveolitis	+
8	11.9	61.8	1.9	Alveolitis	–
9	62.3	84.4	2.0	Alveolitis, Masson body	–
Patient	71.4	41.6	0.8	Alveolitis	–

BALF, bronchoalvelar lavage fluid; TBLB, transbronchial lung biopsy; CD, cluster of differentiation.

specificity. It is possible that the cut-off level should have been much higher. The level of inter-correlation among the biomarkers was the highest between KL-6 and SP-D (r = 0.90). KL-6 was independent of LST, and the measurement of both markers was useful to screen the HP patients in this study. The LST values in BALF were also significantly higher in the HP group (n = 5) than those in the Non-HP group (n = 5). It is possible that LST using BALF was useful to detect the causative antigen. In this study, Enoki and the culture medium were not identified as causative antigens. Interestingly, several participants had high LST values to even Enoki and the culture medium in the same way as *P. citrinum*. The cut-off levels in Enoki and the culture medium were both decided to be 400% because the lowest LST value to each antigen of normal controls was less than 400%.

DIT is also considered to be useful to screen HP patients to identify the cause of HP [Sanderson et al., 1992]. Precipitating lines were observed to *P. citrinum* in all of the HP patients. On the other hand, the line was also observed to *P. citrinum* in one of the 44 non-HP workers, who was working in a different farm where the HP patients worked. The sensitivity and specificity in DIT were both good at 100% and 97.5%, respectively, and DIT was helpful to screen HP patients and find the causative antigen in this study. However, some studies have reported that serum precipitating antibodies provided a useful marker of exposure to antigens but were not specific to an allergic respiratory

TABLE V. LST in Serum and BALF

	Enoki		Culture medium		*Penicillium citrinum*		
Case no.	Serum (%)	BALF (%)	Serum (%)	BALF (%)	Serum (%)	BALF (%)	Serum precipitins to *Penicillium citrinum*
(A) non-HP (n = 5)							
2	933	91	392	ND	457	112	–
3	572	110	712	ND	584	133	–
4	920	ND	396	ND	677	122	–
5	1,121	164	758	ND	859	582	–
7	1,932	71	1,116	ND	561	66	–
(B) HP (n = 5)							
1	111	240	544	768	1,661	3,518	+
6	263	353	342	ND	967	3,246	+
8	451	84	422	ND	1,696	321	+
9	118	134	263	204	281	1,096	+
Patient	147	300	237	210	715	5,679	+

LST, lymphocyte stimulation test; BALF, bronchoalvelar lavage fluid; ND, not done.
LST value ≥400% is considered to be positive on each antigen.

TABLE VI. The Comparison Among 48 Workers and the First HP Patient

	(A) non-HP (n = 44)	(B) HP (n = 5)	*P*-value (A) versus (B)	Cut-off level	AUC	Se. (%)	Sp. (%)
KL-6 (U/ml)	265.8 ± 136.2	2,790.4 ± 2,607.6	0.0004	450	0.99	100	93.2
SP-A (ng/ml)	31.8 ± 12.5	52.0 ± 33.0	0.0954	43.8	0.73	40	93.2
SP-D (ng/ml)	51.8 ± 29.7	239.6 ± 184.4	0.0037	110	0.90	60	93.2
LST (%)[a]	438.4 ± 547.0	1,064 ± 612.4	0.0105	400	0.87	80	56.8
DIT	1[b]	5[b]				100	97.5

AUC, area under the curve in ROC analysis; Se., sensitivity; Sp., specificity; DIT, double immunodiffusion test.
[a]LST to *Penicillium citrinum* in serum.
[b]Subjects with positive precipitins in sera to *Penicillium citrinum*.

disease such as HP [do Pico et al., 1976; Burrell and Rylander, 1981]. Tanaka et al. [2001] conducted a 3-year follow-up of allergy in workers cultivating Bunashimeji mushrooms, and reported that the positive rate of serum precipitins to the spores in non-HP workers was 30% in 1 year, 93% in 2 years, 94% in 3 years. These mushroom workers were exposed to Bunashimeji spores. It is possible that the difference in the positive rate in DIT occurred due to the exposure duration to antigens and the amount of antigens. Air sampling was not performed by month to month or year to year in this study, so the level of exposure to *P. citrinum* spores that the worker received cannot be assessed in this study. However, it was possible that *P. citrinum* began to grow very rapidly and the Enoki workers were suddenly exposed to large amounts of *P. citrinum* spores.

Penicillium spp. was commonly found indoors, and it is popular common fungi in Enoki farms. Enoki workers might have been exposed to small amounts of *P. citrinum* spores, Enoki spores and the organic matter in the culture medium for many years which were insufficient to develop HP. The workers may have been sensitized by these antigens. In the Enoki farm where the initial HP patient worked, *P. citrinum* was isolated throughout the work environment in places such as an air-conditioner [Yoshikawa et al., 2006]. *Rhizopus stolonifer* and Enoki were also isolated in that environment, but at a very low level. The investigation was not performed at every farm, however 77% of the workers reported seeing "green" fungus more frequently than usual during the study period, and *P. citrinum* was isolated from 54.2% of the workers. These results suggested that most Enoki workers were being exposed to large amounts of *P. citrinum* spores during that period.

According to the Japan Meteorological Agency, it seemed that precipitation in eastern Nagano prefecture during this study period was more than usual. The study area, which is located in eastern Nagano prefecture, is surrounded by mountains on four sides and is very humid all year round. This humid environment is appropriate for mushroom production. The fact that the area had an abundance of precipitation during this study period suggested that the humidity had been higher than usual. High humidity might have promoted the growth of *P. citrinum*. Furthermore, many workers reported that they often found "green" fungus in their work environments after the type of culture medium was changed 3 months earlier. The change of the culture medium might also be connected with the marked growth of *P. citrinum*.

Interestingly, two of 4 new HP patients were working at the same mushroom farm as the initial HP patient was. In this "epidemic" farm, high levels of *P. citrinum* was isolated throughout the work environment, and "green" fungus was found very easily on the culture medium and Enoki mushrooms themselves. Nine workers including the initial HP patient were involved with cultivating Enoki, and all 8 of the workers participated in this survey. Cases 1, 2, 3, and 8 were picked up for further examinations, and finally cases 1 and 8 were regarded as having HP. The rate of disease in this "epidemic" farm was 3/9 (33.3%). It was possible that the amounts of *P. citrinum* spores in the "epidemic" farm were much higher than those in the other farms. However, the differences of the amounts of the spores among Enoki farms cannot be discussed because air sampling was not performed at every Enoki farm.

In conclusion, 4 new cases of HP were found among workers cultivating Enoki mushrooms in this study. KL-6, SP-D, LST, and DIT were useful for detecting HP patients, and KL-6 was considered to be most useful predictor of HP. DIT was useful not only as a predictor of HP but also as a detector of the causative antigen. It appears that unclean indoor environments associated with changes in the weather and the culture medium promoted the rapid growth of *P. citrinum* and this stimulated the outbreak of HP.

REFERENCES

Abe S, Takahashi H. 2000. Surfactant protein A and D as biomarkers of disease activity in diffuse interstitial pneumonia. Nihon Kokyuki Gakkai Zasshi 38:157–165.

Burrell R, Rylander R. 1981. A critical review of the role of precipitins in hypersensitivity pneumonitis. Eur J Respir Dis 62:332–343.

do Pico GA, Reddan WG, Chmelik F, Peters ME, Reed CE, Rankin J. 1976. The value of precipitating antibodies in screening for hypersensitivity pneumonitis. Am Rev Respir Dis 62:451–455.

Fergusson RJ, Milne LJR, Crompton GK. 1984. Penicillium allergic alveolitis: Faulty instillation of central heating. Thorax 39:294–298.

Holland P, Mauer AM. 1964. Drug induced *in vitro* stimulation of peripheral lymphocytes. Lancet 1:1368–1369.

Kobayashi J, Kitamura S. 1995. KL-6: A serum marker for interstitial pneumonia. Chest 108:311–315.

Kohno N, Kyoizumi S, Awaya Y, Fukuhara H, Yamakido M, Akiyama M. 1989. New serum indicator of interstitial pneumonitis activity: Sialylated carbohydrate antigen KL-6. Chest 96:68–73.

Kubo K, Hanaoka M, Hayano T, Miyahara T, Hachiya T, Hayasaka M, Koizumi T, Fujimoto K, Kobayashi T, Honda T. 1998. Inflammatory cytokines in BAL fluid and pulmonary hemodynamics in high altitude pulmonary edema. Respir Physiol 111:301–310.

Licorish K, Novey HS, Kozak P, Fairshter RD, Wilson AF. 1985. Role of *Alternaria* and *Penicillium* spores in the pathogenesis of asthma. J Allergy Clin Immunol 76:819–825.

Matsui S, Nakazawa T, Umegae Y, Mori M. 1992. Hypersensitivity pneumonitis induced by Shiitake mushroom spores. Intern Med 31:1204–1206.

Mori S, Nakagawa-Yoshida K, Tsuchihashi H, Koreeda Y, Kawabata M, Nishiura Y, Ando M, Osame M. 1998. Mushroom worker's lung resulting from indoor cultivation of Pleurotus osteatus. Occup Med 48:465–468.

Nakagawa-Yoshida K, Ando M, Etches R, Dosman J. 1997. Fatal cases of farmer's lung in a Canadian family. Probable new antigens, *Penicillium brevicompactum and P olivicolor*. Chest 111:245–248.

Nakazawa T, Tochigi T. 1989. Hypersensitivity pneumonitis due to mushroom (*Pholiota nameko*) spores. Chest 95:1149–1151.

Ouchterlony O. 1957. Diffusion-in-gel methods for immunological analysis. Progr Allergy 5:1–78.

Park HS, Jung KS, Kim SO, Kim SJ. 1994. Hypersensitivity pneumonitis induced by *Penicillium expansum* in a home environment. Clin Exp Allergy 24:383–385.

Pit JI. 1998. A laboratory Guide to common *Penicillium* species, commonwealth scientific and industrial research organization, *North Ryde, NSW*, Australia.

Saikai T, Tanaka H, Fuji M, Sugawara H, Takeya I, Tsunematsu K, Abe S. 2002. Hypersensitivity pneumonitis induced by the spore of *Pleurotus Eryngii* (Eringi). Intern Med 41:571–573.

Sanderson W, Kullman G, Sastre J, Olenchock S, O'Campo A, Musgrave K, Green F. 1992. Outbreak of hypersensitivity pneumonitis among mushroom farm workers. Am J Ind Med 22:859–872.

Solley GO, Hyatt RE. 1980. Hypersensitivity pneumonitis induced by *Penicillium* species. J Allergy Clin Immunol 65:65–70.

Steawart CJ. 1974. Mushroom worker's lung-two outbreaks. Thorax 29:252–257.

Tanaka H, Sugawara H, Saikai T, Tsunematsu K, Takahashi H, Abe S. 2000. Mushroom worker's lung caused by spores of *Hypsizigus marmoreus* (Bunashimeji). Chest 118:1506–1509.

Tanaka H, Saikai T, Sugawara H, Tsunematsu K, Takeya I, Koba H, Matsuura A, Imai K, Abe S. 2001. Three-year follow-up study of allergy in workers in a mushroom factory. Respir Med 95:943–948.

Tsushima K, Fujimoto K, Yamazaki Y, Takamizawa A, Amari T, Koizumi T, Kubo K. 2001. Hypersensitivity pneumonitis induced by spores of *Lyophyllum aggregatum*. Chest 120:1085–1093.

Tsushima K, Fujimoto K, Yoshikawa S, Kawakami S, Koizumi T, Kubo K. 2005. Hypersensitivity pneumonitis due to Bunashimeji mushroom in the mushroom industry. Int Arch Allergy Immunol 137:241–248.

Van den Bogart HG, Van den Ende G, Van Loon PC, Van Griensven LJ. 1993. Mushroom worker's lung: Serologic reactions to thermophilic actinomycetes present in the air of compost tunnels. Mycopathologia 122:21–28.

Wei DL, Chen JH, Jong SC, Shen HD. 1993. Indoor airborne *Penicillium* species in Taiwan. Curr Microbiol 26:485–490.

Yoshikawa S, Tsushima K, Koizumi T, Kubo K, Kumagai T, Yamazaki Y. 2006. Hypersensitivity pneumonitis by spores of *Penicillium citrinum* in the workers cultivating *Enoki* mushroom. Intern Med 45:537–541.

Yokoyama A, Kohno N, Kondo K, Ueda S, Hirasawa Y, Watanabe K, Takada Y, Hiwada K. 1998. Comparative evaluation of sialylated carbohydrate antigens, KL-6, CA19-9 and SLX as serum markers for interstitial pneumonia. Respirology 3:199–202.

研究成果の刊行に関する一覧表
平成19年度　国立病院機構　東京病院　呼吸器科　永井英明

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Haruyuki Ariga, Yoshiko Kawabe, Hideaki Nagai, Atsuyuki Kurashima, Kimiihiko Masuda, Hirotoshi Matsui, Atsuhisa Tamura, Naohiro Nagayama, Shinobu Akagawa, Kazuko Machida, Akira Hebisawa, Yutsuki Nakajima, Hideki Yotsumoto, and Toru Mori	Diagnosis of Active Tuberculous Serositis by Antigen-Specific Interferon-γ Response of Cavity Fluid Cells	CID	45	1559-1567	2007
永井英明	HIV合併結核の現状と展望	呼吸器科	11	423-427	2007
永井英明、川辺芳子、有賀晴之、鴫山文子、島田昌裕、久能木真喜子、松井芳憲、川島正裕、鈴木純子、大島信治、益田公彦、松井弘稔、田村厚久、長山直弘、赤川志のぶ、町田和子、倉島篤行、四元秀毅	HIV感染症における結核感染診断に対してのQuantiFERON-TB第２世代の有用性についての検討	結核	82	635-640	2007
永井英明	[ICTがおさえておきたい感染症対策2007] おさえておきたい結核	INFECTION CONTROL	16	938-943	2007
永井英明	[今日の結核症－的確な診断から治療へ] 結核症の治療 合併症等を有する特殊な症例の治療	診断と治療	95	1973-1980	2007

Diagnosis of Active Tuberculous Serositis by Antigen-Specific Interferon-γ Response of Cavity Fluid Cells

Haruyuki Ariga,[1] Yoshiko Kawabe,[1] Hideaki Nagai,[1] Atsuyuki Kurashima,[2] Kimihiko Masuda,[1] Hirotoshi Matsui,[1] Atsuhisa Tamura,[1] Naohiro Nagayama,[1] Shinobu Akagawa,[1] Kazuko Machida,[1] Akira Hebisawa,[3] Yutsuki Nakajima,[4] Hideki Yotsumoto,[1] and Toru Mori[5]

Departments of [1]Respiratory Medicine, [2]Clinical Research, [3]Pathology, and [4]Thoracic Surgery, National Hospital Organization, Tokyo National Hospital, and [5]Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan

Background. To develop a more accurate methodology for diagnosing active tuberculous pleurisy, as well as peritonitis and pericardits of tuberculous origin, we established an antigen-specific interferon γ (IFN-γ)–based assay that uses cavity fluid specimens.

Methods. Over a 19-month period, 155 consecutive, nonselected patients with any cavity effusion were evaluated. Study subjects were 28 patients with bacteriologically confirmed active tuberculous serositis and 47 patients with definitive nontuberculous etiology. Culture was performed for 18 h with fluid mononuclear cells in the supernatant of the effusion together with saline or *Mycobacterium tuberculosis*–specific antigenic peptides, early secretory antigenic target 6 and culture filtrate protein 10. IFN-γ concentrations in the culture supernatants were measured.

Results. In patients with active tuberculous serositis, antigen-specific IFN-γ responses of cavity fluid samples were significantly higher than those of nontuberculous effusion samples. Area under the receiver operating characteristic (AUROC) curve was significantly greater for cavity fluid IFN-γ response (AUROC curve, 0.996) than for cavity fluid adenosine deaminase and whole-blood IFN-γ responses (AUROC curve, 0.882 and 0.719, respectively; $P = .037$ and $P < .001$, respectively). Although the AUROC curve was greater for cavity fluid IFN-γ response than for background cavity fluid IFN-γ level (AUROC curve, 0.975), the AUROC curves were not statistically significantly different ($P = .74$). However, multivariate logistic regression analysis revealed that cavity fluid IFN-γ responses were significantly associated with the diagnosis, even after adjustment for background IFN-γ level (adjusted odds ratio, 1.21; 95% confidence interval, 1.03–1.42; $P < .001$).

Conclusions. The cavity fluid IFN-γ assay could be a method for accurately and promptly diagnosing active tuberculous serositis.

Tuberculosis is a serious infectious disease that threatens the health of mankind, even in the modern world [1]. Active tuberculous pleuritis, peritonitis, and pericarditis should always be considered as possible causes of cavity fluid in the daily practice of clinical medicine. Their definitive diagnosis is based on the identification of *Mycobacterium tuberculosis* after culture of effusion or tissue biopsy specimens. However, the rate of positive results of such cultures of pleural fluid specimens is not high (<30%) [2]. In addition, because bacteriological results cannot be obtained rapidly, antituberculous agents should, in some cases, be instituted on the basis of clinical speculation without waiting for the definitive diagnosis. Measurement of nonspecific markers, such as adenosine deaminase (ADA) and IFN-γ, in the supernatant of fluid specimens has been used to diagnose pleural tuberculosis [3, 4]. A meta-analysis concluded that maximum joint sensitivity and specificity was 93% for the ADA assay and 96% for the IFN-γ assay [5]. It has been reported that the levels of these markers are also increased in the context of other diseases [6–9], and there is some contention as to the use of these tests [10, 11]. Thus, it would be desirable to develop a more specific diagnostic method for active tuberculous serositis (ATBS).

Active tuberculous pleuritis is thought to be caused by the spread of a small subpleural or hilar lymph node

Received 1 May 2007; accepted 2 August 2007; electronically published 8 November 2007.

Reprints or correspondence: Dr. Haruyuki Ariga, Dept. of Respiratory Medicine, Tokyo National Hospital, 3-1-1 Takeoka, Kiyose, Tokyo 204-8585, Japan (arigah-in@tokyo-hosp.jp).

Clinical Infectious Diseases 2007; 45:1559–67

1058-4838/2007/4512-0005$15.00
DOI: 10.1086/523591

caseous focus of *M. tuberculosis* into the cavity, and T cells that specifically respond to tuberculous antigens and produce IFN-γ are recruited in the pleural cavity of patients with active tuberculous pleuritis [12, 13]. *M. tuberculosis*–specific protein antigens early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), which are absent from the genomes of all bacille Calmette-Guérin substrains and most nontuberculous mycobacteria, have been identified [14]. A specific method for the detection of tuberculous infection has been developed in which IFN-γ production (the number of IFN-γ–producing cells) is measured after stimulation of peripheral blood with these antigens in vitro [15–19]. We hypothesized that, when cavity fluid mononuclear cells of patients with ATBS were stimulated with the antigenic peptides ESAT-6 or CFP-10, IFN-γ responses that are greater than those achieved using the stimulation of peripheral blood might be expected. We report our results, which suggest the possibility of accurate diagnostic procedures for ATBS that reflect the local immune response in the cavity.

PATIENTS AND METHODS

Patients and evaluation of their cavity effusion specimens. Over a 19-month period from November 2004 through June 2006, 155 consecutive, nonselected patients with any cavity effusion were evaluated at the inpatient department of Tokyo National Hospital (Tokyo, Japan). Either cavity centesis or endoscopic procedures, including thoracoscopic and perioteneoscopic procedures, was performed for all patients; 75 patients for whom the etiology of cavity effusion could be definitively identified were enrolled. Table 1 shows the profiles and the clinical diagnoses that these patients received. All patients with tuberculosis had received <7 days of antituberculous chemotherapy at the time of testing. None of these patients had conditions, such as HIV infection, associated with severe immunocompromise, and none were receiving immunosuppressive drugs.

The differential diagnosis based on the evaluation of the pleural effusion specimens was made using the following criteria. A definitive diagnosis of active tuberculous pleuritis, peritonitis, or pericarditis was made on the basis of positive culture results and identification of *M. tuberculosis* in cavity effusion or parietal tissue specimens. With regard to nontuberculous pleuritis, peritonitis, and pericarditis, the diagnosis of neoplastic pleuritis, peritonitis, or pericarditis was based on detection of malignant cells in pleural effusion or tissue specimens; parapneumonic effusion was defined by the presence of fever, spu-

Table 1. Profiles of patients with cavity effusion.

Variable	Patients with active tuberculous serositis (n = 28)	Patients with nontuberculous effusion (n = 47)	All (n = 75)
Age, years			
Mean ± SD	60.5 ± 22	72.1 ± 10.8	67.9 ± 17.1
Range	20–91	46–91	20–91
Sex			
Male	24 (86)	40 (85)	65 (87)
Female	4 (14)	7 (15)	10 (13)
Clinical diagnosis			
Active tuberculous pleuritis	26	0	...
Active tuberculous peritonitis	1	0	...
Active tuberculous pericarditis	1	0	...
Nontuberculous pleuritis	0	45	...
Neoplastic pleuritis	0	24	...
Lung cancer	0	22	...
Malignant mesothelioma	0	2	...
Parapneumonic effusion	0	12	...
Heart failure	0	5	...
Asbestos pleural effusion	0	1	...
Posttraumatic pleural effusion	0	1	...
Mycobacterium avium pleuritis	0	1	...
Hypothyroidism (pleural effusion)	0	1	...
Cancerous peritonitis	0	1	...
Cancerous pericarditis	0	1	...

NOTE. Data are no. or no. (%) of patients, unless otherwise indicated.

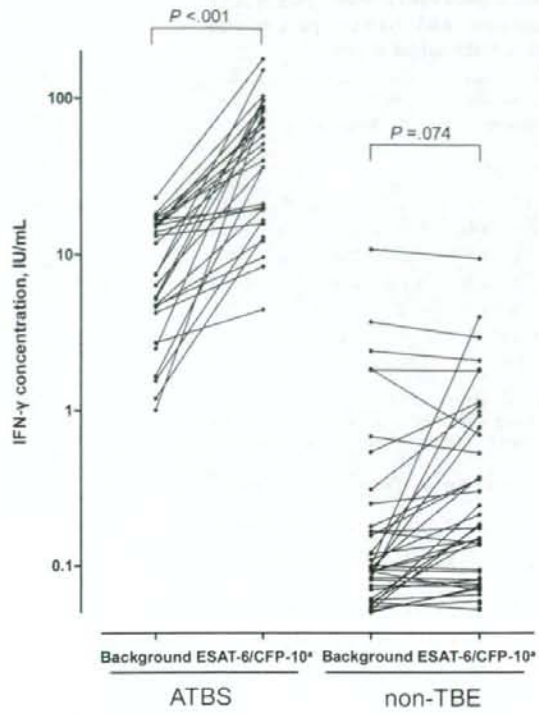

Figure 1. IFN-γ production in response to stimulation with *Mycobacterium tuberculosis*–specific antigen. Pleural, peritoneal, and pericardial fluid cells obtained from patients with active tuberculous serositis (ATBS) or nontuberculous effusion (non-TBE) were harvested and suspended in the individual supernatant of the effusion at 1–5 million cells/mL, stimulated with saline or *M. tuberculosis*–specific antigens, and incubated for 18 h, after which IFN-γ concentrations in the culture supernatant were measured by ELISA. If, in an initial assay, a supernatant was found to contain >15 IU/mL of IFN-γ, the specimen was diluted with zero standard and reassayed. IFN-γ concentrations in each patient, which were expressed as background saline control IFN-γ concentrations and concentrations after stimulation with specific antigens (early secretory antigenic target 6 [ESAT-6] and culture filtrate protein 10 [CFP-10]), are shown as closed circles connected with lines. Student's *t* test was used for differences between the ATBS and non-TBE groups. IFN-γ values after antigen stimulation are the measured values, not values subtracted from the corresponding background control values. ªThe higher IFN-γ concentrations after stimulation with either antigenic peptides ESAT-6 or CFP-10 are represented.

tum, infiltrates on chest radiograph, an infectious inflammatory response detected by serological testing, and improvement after the administration of antibiotics. Heart failure was assigned as the cause of pleural effusion if cardiac dysfunction was evident on ultrasonographic cardiograph and improvement was noted after the administration of diuretics or cardiotonic drugs. Asbestos pleural effusion was diagnosed on the basis of detection of asbestos particles or fibrous patches in pleural biopsy specimens. Posttraumatic hemothorax was indicated when there was evident pooling of blood in the thorax after chest trauma. Pleuritis due to *Mycobacterium avium* was considered on the basis of identification of *M. avium* in pleural effusion specimens.

Stimulation of cavity fluid cells with* M. tuberculosis*–specific antigens (cavity fluid IFN-γ assay). Twenty to 50 mL of cavity effusion were centrifuged at 2000 rpm for 15 min. The supernatant was separated, and the number of nucleated cells in the cell compartment was determined. Cells were then suspended at 1–5 million cells/mL in the individual supernatant of the effusion. One milliliter of this cell suspension was then added to 4 wells of 24-well plates (NUNC) and underwent culture in the presence of *M. tuberculosis* antigen peptides ESAT-6 or CFP-10 at a final concentration of 1 μg/mL (QuantiFERON-TB 2G; Cellestis) [19]. To the remaining 2 wells, physiological saline was added instead of the antigens, because a measurement of background IFN-γ of cavity fluid and phytohemagglutinin (5 μg/mL) was used as a positive control. Cells were incubated at 37°C for 18 h in a 5% CO_2 incubator, and the cultured supernatant was harvested for measurement of IFN-γ concentration. The value of the cavity fluid assay was defined as the difference between the determined higher value after stimulation with either ESAT-6 or CFP-10 and the value of the measurement of background IFN-γ. The whole-blood IFN-γ assay was performed as previously described [19].

Measurement of IFN-γ and ADA concentrations. IFN-γ concentration was measured using the QuantiFERON-CMI ELISA (Cellestis) according to procedures described in the manufacturer's instructions. The IFN-γ values were expediently replaced with 0.05 when the difference between the antigen-stimulated level and the background level was negative or <0.05 IU/mL (lower limit of measurement). If, in an initial assay, the supernatant was found to contain >15 IU/mL of IFN-γ (upper limit of measurement), the specimen was diluted with zero standard and reassayed. When both stimulated and background IFN-γ concentrations were extremely high, we performed another culture using plasma samples from each patient for medium to decrease the background IFN-γ concentration. The result of the whole-blood assay was considered to be indeterminate if the value for the positive control sample was <0.5 IU/mL after subtraction of the value for the negative control sample. ADA concentration in the supernatant of cavity fluid samples was measured by AUTO A MIZUHO ADA (Mizuho Medy). Three technicians with expertise in laboratory work were blinded to the clinical information of the patients, results of other assays in this study, and the reference standard.

Statistical analysis. Univariate analyses were performed using Student's *t* test and the Mann-Whitney *U* test. We constructed a receiver operating characteristic (ROC) curve by

Table 2. IFN-γ response in cavity fluid and whole-blood cells to tuberculous-specific and nonspecific antigens and adenosine deaminase (ADA) levels in patients with active tuberculous serositis and patients with nontuberculous effusion.

Variables	Patients with active tuberculous serositis (n = 28)	Patients with nontuberculous effusion (n = 47)	P
Background IFN-γ level in cavity fluid	7.4 (4.5–15.2)	0.09 (0.05–0.16)	<.001
ESAT-6–background[a]	26.4 (6.8–49.4)	0.05 (0.05–0.08)	<.001
CFP-10–background[b]	12.2 (2.8–37.7)	0.05 (0.05–0.09)	<.001
ESAT-6 or CFP-10–background[c]	34.8 (7.3–74.3)	0.05 (0.05–0.19)	<.001
Mitogen–background[d]	22.6 (2.1–55.1)	6.90 (0.32–21.5)	.009
Whole-blood IFN-γ assay result[e]	0.54 (0.3–0.9)	0.07 (0.05–0.54)	<.001
ADA concentration[f]	61.3 (43.0–71.5)	13.80 (7.95–27.5)	<.001

NOTE. Data are median value (interquartile range). Mann-Whitney U test was used for differences in IFN-γ and ADA concentrations between the patients with active tuberculous serositis and patients with nontuberculous effusion. CFP-10, culture filtrate protein 10; ESAT-6, early secretory antigenic target 6.

[a] Difference between the determined IFN-γ concentration after stimulation with ESAT-6 and the background IFN-γ concentration in cavity fluid.

[b] Difference between the determined IFN-γ concentration after stimulation with CFP-10 and the background IFN-γ concentration in cavity fluid.

[c] Difference between the determined higher IFN-γ concentration after stimulation with either ESAT-6 or CFP-10 and the background IFN-γ concentration.

[d] Difference between the determined IFN-γ concentration after stimulation with mitogen and the background IFN-γ concentration in cavity fluid.

[e] None of the patients had indeterminate results, and 1 patient with tuberculosis was unavailable.

[f] Not performed for 1 patient with tuberculosis.

plotting the rate of sensitivity against the rate of false-positive results over a range of cutoff values of IFN-γ and ADA for the assessment of diagnostic accuracy. Area under the ROC curve was calculated using the trapezoidal rule. Standard errors of the areas and comparisons between assays were calculated using a method described elsewhere [20]. Pairwise comparisons were made between area under the ROC curve for the cavity fluid IFN-γ assay and that for each of the following measures: background IFN-γ and ADA assays and the whole-blood IFN-γ assay. Adjustment for multiple comparisons was made using the Dunn-Sidák method. To assess whether the cavity fluid assay lends additional diagnostic information to that provided by the background IFN-γ level, these 2 measurements were introduced as predictors in a multivariate logistic regression model. These 2 explanatory variables were treated as continuous variables. $P < .05$ was considered to be statistically significant. All statistical analyses were performed using Stata, version 9 (Stata).

This study was approved by the ethics review committee of our hospital. We obtained informed consent from all participants in the study.

RESULTS

The mean age of patients with nontuberculous effusion (non-TBE) was significantly higher than that of patients with ATBS (72.4 years vs. 60.5 years; $P = .013$). There was no statistically significant difference in sex between the groups (85% male vs. 81% male; $P = .745$) (table 1).

Figure 1 shows results of the cavity fluid IFN-γ assay for each subject (in both the ATBS and non-TBE groups), including background IFN-γ values and the highest values for either of the stimulation antigens, ESAT-6 or CFP-10. The IFN-γ concentration was significantly increased after antigen stimulation, compared with the background IFN-γ concentration, in every patient with ATBS ($P < .001$). However, in patients with non-TBE, IFN-γ concentrations did not significantly increase after stimulation with antigens ($P = .074$)

The median values and the interquartile range of background cavity fluid IFN-γ, antigen-specific cavity fluid IFN-γ, whole-blood, and ADA assays are shown in table 2. Individual values for these parameters, as well as the result of the cavity fluid assay multiplied by the background IFN-γ level, are indicated by dots in figure 2. Background IFN-γ concentrations were significantly higher in patients with ATBS than in patients with non-TBE. Cavity fluid IFN-γ responses to both ESAT-6 and CFP-10 were significantly greater for patients with ATBS than for patients with non-TBE. Likewise, when an IFN-γ response was represented by a higher value of response to either ESAT-6 or CFP-10, the difference between the values for the ATBS group and the non-TBE group was greater. IFN-γ responses to mitogen were significantly greater for patients with ATBS than

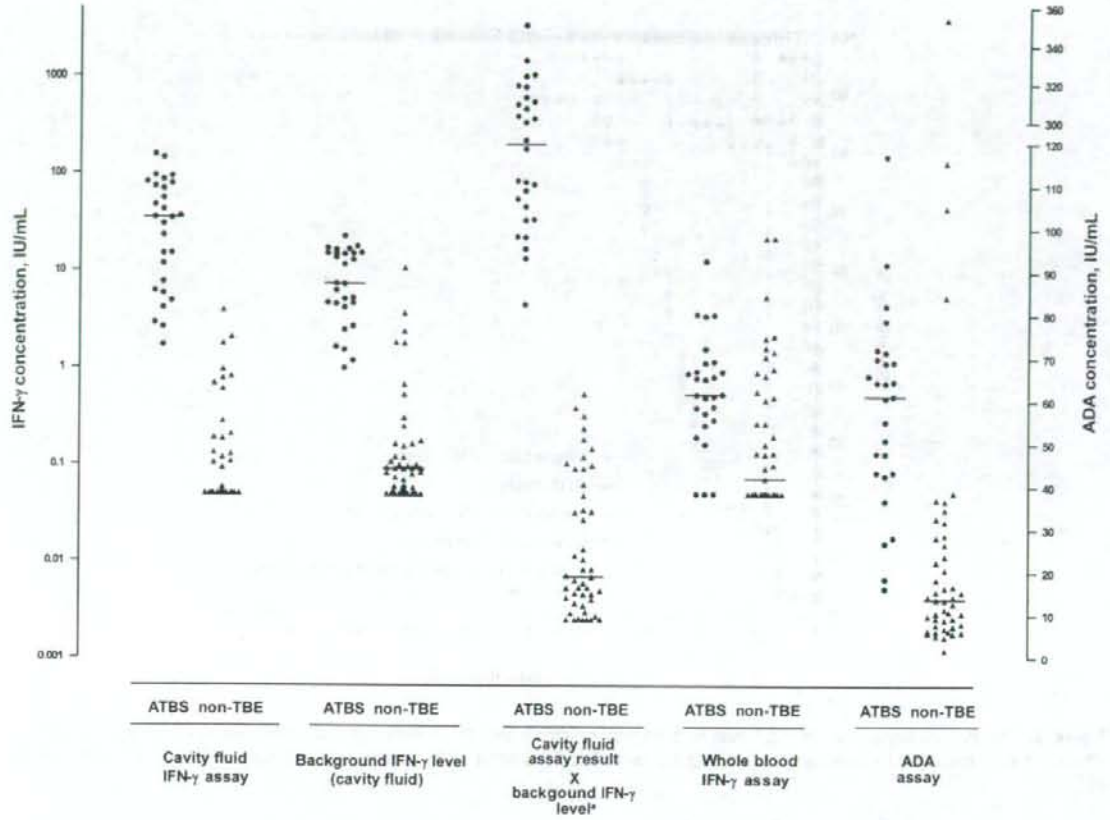

Figure 2. IFN-γ concentrations in cavity fluid and whole-blood and adenosine deaminase (ADA) concentrations in patients with active tuberculous serositis (ATBS) and in patients with nontuberculous effusion (non-TBE). Individual results of whole-blood IFN-γ, ADA, cavity fluid IFN-γ, and background control IFN-γ assays for patients with ATBS or non-TBE are shown. IFN-γ values are represented by the higher value of response to either early secretory antigenic target 6 (ESAT-6) or culture filtrate protein 10 (CFP-10) after subtraction of each background control IFN-γ value for the cavity fluid and whole-blood IFN-γ assays. Values for patients with ATBS and non-TBE are represented by closed circles and triangles, respectively. Horizontal lines in the columns represent the median value in each group. *Values calculated by multiplying the result of the cavity fluid IFN-γ assay by the background cavity fluid IFN-γ level and expressed as IU/mL^2.

for those with non-TBE. The median value of IFN-γ in the whole-blood IFN-γ assay was significantly higher for patients with ATBS than for patients with non-TBE, as was the median ADA value. For the whole-blood assay, none of the patients had indeterminate results, and 1 patient with ATBS was unavailable for testing. The ADA assay was not performed for 1 patient with tuberculosis.

Relative discriminative accuracy of the whole-blood, ADA, background IFN-γ, and cavity fluid assays was assessed in the area under the ROC curve (figure 3, table 3). The relative discriminative accuracy of the cavity fluid assay was statistically significantly different from that of the ADA ($P = .037$) and whole-blood assays ($P < .001$). The ROC curve for the cavity fluid assay was bowed further to the upper left, compared with that for the background IFN-γ level, but the areas under the ROC curve for the 2 assays were not statistically significantly different at the 5% level of type I error rate ($P = .74$).

Multivariate logistic regression was used as an aid in assessment of whether the cavity fluid assay added any diagnostic information to that yielded by the background IFN-γ level (table 4). Spearman's coefficient of correlation between the 2 measurements was 85%. The 2 measurements did not display statistical evidence of an interaction ($P = .84$). The Hosmer-Lemeshow test did not indicate a lack of fit ($P = .74$); thus, the predictors provide for a reasonably well calibrated model without data transformation. The cavity fluid assay displayed

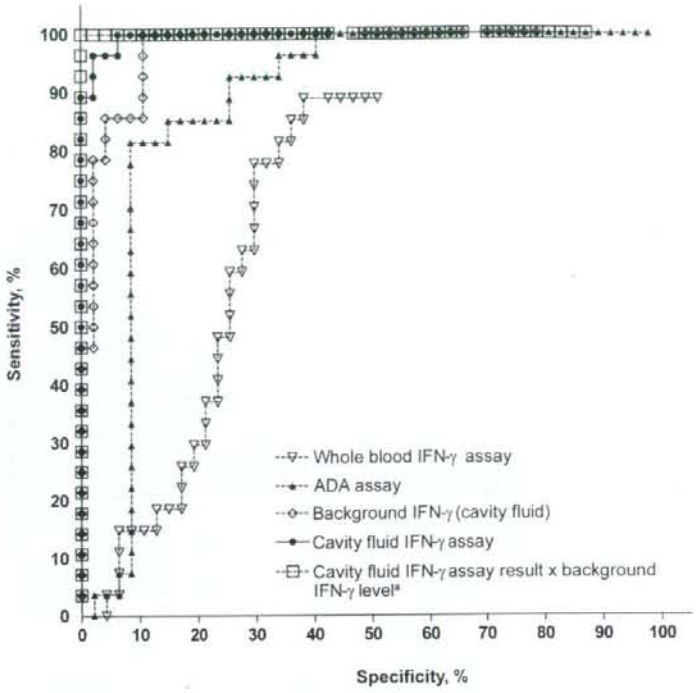

Figure 3. Receiver operating characteristic curves for 5 diagnostic methods used for patients with active tuberculous serositis and nontuberculous effusion. [a]Values calculated by multiplying the result of the cavity fluid IFN-γ assay by the background cavity fluid IFN-γ level and expressed as IU/mL[2].

a statistically significantly nonzero level of association with diagnostic status, even after adjustment for background IFN-γ concentrations, which supports the contention that the cavity fluid assay provides for improved diagnostic accuracy over that provided by the background IFN-γ level alone.

The cutoff value for each test was chosen to maximize specificity without significant loss of sensitivity. Table 5 shows sensitivity, specificity, likelihood ratio, and predictive value by cross classification. To calculate the predictive value, ~3.2% of pretest probability of ATBS in our specialist hospital for tuberculosis was used. As a result, both the likelihood ratio and predictive value of the cavity fluid assay were greater than those of the other assays. Furthermore, the value from when the results of the cavity fluid assay were multiplied by the background IFN-γ level was the most sensitive and specific predictor of ATBS.

DISCUSSION

Area under the ROC curve is the primary index for assessing the discriminative accuracy of a diagnostic method. Using this index, the cavity fluid assay displayed greater ability to discriminate ATBS than did either the ADA or whole-blood assays. The ROC curve index was not able to detect a difference in discriminative accuracy between unstimulated and specific antigen-stimulated IFN-γ response ex vivo. Nevertheless, a diagnostic advantage in assaying specific antigen-stimulated IFN-γ response was evident such that a multivariate logistic regression model provides a better fit to the clinical diagnosis when specific antigen-simulated IFN-γ production is included; the regression coefficient for the cavity fluid IFN-γ assay is statistically significantly different from zero. This better fit indicates improved calibration of the model. It is recognized that

Table 3. Comparison of diagnostic accuracy by area under the receiver operating characteristic (AUROC) curves.

Variable	AUROC curve (95% CI)	SE
Whole-blood IFN-γ assay	0.719 (0.598–0.838)	6.1
ADA assay	0.882 (0.799–0.965)	4.2
Background IFN-γ of cavity fluid	0.975 (0.946–1.004)	1.5
Cavity fluid IFN-γ assay[a]	0.996 (0.989–1.004)	0.4

[a] The value of the cavity fluid IFN-γ assay was defined as the difference between the determined higher IFN-γ value after stimulation with either early secretory antigenic target 6 or culture filtrate protein 10 and the background IFN-γ concentration.

Table 4. Multivariate logistic regression analysis.

Variable	Regression coefficient, log odds per IU/mL	SE	Likelihood ratio[a]	*P*	OR (95% CI)
Intercept	−3.47	0.79	...	...	
Background IFN-γ of cavity fluid	0.34	0.23	3.10	.08	1.40 (0.80–2.19)
Cavity fluid IFN-γ assay	0.19	0.08	18.98	<.005	1.21 (1.03–1.42)

NOTE. Two explanatory variables were treated as continuous variables. Standardized ORs (each variable scaled to its interquartile range) were 5.6 for background IFN-γ and 55 for cavity fluid IFN-γ assay. Spearman's coefficient of correlation between the 2 measurements was 85%. The 2 measurements did not display statistical evidence of an interaction ($P = .84$). The Hosmer-Lemeshow test did not indicate a lack of fit ($P = .74$); thus, the predictors provide for a reasonably well calibrated model without data transformation.

[a] Likelihood ratio was determined using χ^2 test statistics and was single-degree-of-freedom.

area under the ROC curve is important but not always optimum in assessment of diagnostic tests, especially for predictive assessment of risk [21].

For the IFN-γ assay using cavity fluid, the IFN-γ concentration was significantly increased after antigen stimulation, compared with the background IFN-γ concentration, for every patient with ATBS (figure 1). It may be speculated that this result was because of further IFN-γ production by antigen-specific T cells in response to stimulation. We have obtained similar results for 30 patients with pleural effusion in whom active tuberculous pleuritis was strongly suspected clinically. Although, in these patients, definitive bacteriological evidence could not be obtained from pleural effusion specimens, antituberculous chemotherapy was effective (data not shown). In contrast, for the majority of 47 patients with effusion of nontuberculous etiology, IFN-γ concentrations did not increase after stimulation with antigens. This was also true for 5 of 47 patients with nontuberculous pleuritis who had relatively high background IFN-γ concentrations (>1 IU/mL).

In 3 of the patients with non-TBE who had a history of prior tuberculosis and in some of those without any clinical history of tuberculosis, IFN-γ concentrations were slightly increased in cavity fluid after stimulation, compared with background IFN-γ concentrations. Similarly, results of the whole-blood assay were positive for all of these patients. It may be speculated that when patients with a history of tuberculosis encounter serositis attributable to causes other than tuberculosis, preexisting antigen-specific memory T cells in peripheral blood also appear in the effusion and produce IFN-γ through stimulation by antigens in vitro. If diagnostic evaluation was made only on the basis of measuring an antigen-specific IFN-γ response or on a count of IFN-γ–producing cells by enzyme-linked immunospot assay [22], there is a possibility that some patients with an effusion of nontuberculous etiology who have incidental latent tuberculosis infection (LTBI) will receive a false-positive diagnosis. In such cases, non-TBE with LTBI can be differentially diagnosed from ATBS on the basis of low background IFN-γ concentration (figure 1). We speculate that high background IFN-γ concentrations in patients with ATBS are a reflection of the ongoing active status of the local type I helper T cellular immune response. High IFN-γ concentrations in the supernatant of pleural effusion specimens from patients with tuberculous pleuritis have been reported [3, 7, 23, 24]. However, it has also been demonstrated that the IFN-γ concentration can be high in those patients with an effusion of nontuberculous etiology [7]. In our study, 5 of 47 patients with non-TBE (i.e., *M. avium* pleuritis, cancerous pleuritis, and parapneumonia) had background IFN-γ concentrations that were similar to those found in patients with ATBS (>1 IU/mL). Thus, it appears that specificity of the background IFN-γ level may be limited and that false-positive responses can occur. The cavity fluid assay can assess both background IFN-γ and antigen-specific IFN-γ responses simultaneously, and it is possible to compensate for the fault mutually by assessing the value of both responses together. We demonstrated that the values from when the result of the cavity fluid assay was multiplied by the result of the background IFN-γ possibly could be used to accurately diagnose active tuberculous effusion (figure 2, table 5).

Although both the enzyme-linked immunospot assay using the peripheral blood and whole-blood IFN-γ assay have been reported for the diagnosis of tuberculosis, the results of these assays were also positive for people with LTBI. The whole-blood assay also has been reported to be highly specific for *M. tuberculosis* infection, but it cannot discriminate between active tuberculosis and LTBI. In our study, the group of patients who did not have ATBS likely contained a number of subjects with LTBI. Indeed, 3 patients with non-TBE who had a documented history of prior tuberculosis had positive whole-blood assay results. Similarly, 11 other patients who did not have ATBS had positive whole-blood assay results but had no clear history of tuberculosis or evidence of an old tuberculous lesion on chest radiograph (table 5). Because of the high specificity (>98%) of the whole-blood IFN-γ assay among a young population with no risk for tuberculosis [19], we speculated that these patients were latently infected with tuberculosis, reflecting their age and an era when tuberculosis was prevalent in Japan. An additional

Table 5. Comparison of diagnostic accuracy of adenosine deaminase (ADA), whole-blood, and cavity fluid IFN-γ assays.

Variable	Cutoff value	Result, positive: negative		Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive likelihood ratio	Negative likelihood ratio	PPV[a]	NPV[a]
		ATBS group	Non-TBE group						
Whole-blood IFN-γ assay	0.281	21:6	14:33	77.8 (57.7–91.4)	70.2 (55.1–82.7)	2.6	3.2	7.94	99.0
ADA assay	40.700	22:5	4:43	81.5 (61.9–93.7)	91.5 (79.6–97.6)	9.6	4.9	24.5	99.3
Background IFN-γ of cavity fluid	2.456	24:4	2:45	85.7 (67.3–96.0)	95.6 (85.5–99.5)	20.1	6.7	39.7	99.5
Cavity fluid IFN-γ assay[b]	2.352	27:1	1:46	96.4 (81.7–99.9)	97.8 (88.7–99.95)	45.3	27.4	60.3	99.9
Cavity fluid assay result times the background IFN-γ level[c]	2.590	28:0	0:47	100 (87.7–100)	100 (92.5–100)	...	...	100.0	100.0

NOTE. For the whole-blood assay, none of the patients had indeterminate results, and 1 patient with active tuberculous serositis (ATBS) was unavailable. The ADA assay was not performed for 1 patient with ATBS. Non-TBE, nontuberculous effusion; NPV, negative predictive value; PPV, positive predictive value.

[a] Pretest probability of active tuberculous effusion in our department of respiratory medicine was 3.2%

[b] The result of the cavity fluid IFN-γ assay was defined as the difference between the determined higher value of IFN-γ after stimulation with either early secretory antigenic target 6 or culture filtrate protein 10 and the background IFN-γ concentration.

[c] The values from when the result of the cavity fluid IFN-γ assay was multiplied by the background IFN-γ level.

finding was that the whole-blood assay result was negative for 6 of 27 patients with ATBS. In this study, results of the whole-blood assay showed reduced sensitivity (77.8%), compared with the sensitivity found in previous reports (89%) [19]. It has been reported that T cells that are specific for tuberculous antigens are sequestered from the circulation to the pleural cavity in patients with pleural tuberculosis [22]. The migration of antigen-specific T cells from peripheral blood to the active site of disease was thought to be 1 possible cause of lower than previously reported sensitivity of the whole-blood assay.

ADA in the supernatant of pleural effusion is reported as a diagnostic marker for tuberculous pleuritis [25–27]. The sensitivity and specificity of the ADA assay were reported to be 47.1%–100% and 0%–100%, respectively [28]. In our study, ADA concentrations were significantly higher in patients with ATBS than in patients with non-TBE. With regard to both sensitivity and specificity, the ADA assay had better performance than did the whole-blood assay for diagnosis of ATBS (table 5). However, in 4 of 47 patients with non-TBE (attributable to asbestosis, adenocarcinoma, and parapeumonic pleural effusion), ADA concentrations were as high as those in patients with ATBS. Furthermore, in some patients with active pleural and peritoneal tuberculosis, the ADA concentration was as low as that in patients with non-TBE. These false-positive and false-negative cases will be problematic when physicians make decisions regarding the initiation of long-term antituberculous chemotherapy.

In conclusion, we reported a highly sensitive and specific diagnostic method for active tuberculous pleuritis, tuberculous peritonitis, and pericarditis in which IFN-γ responses were measured after stimulation of cavity fluid cells with *M. tuberculosis*–specific antigens. The cavity fluid IFN-γ assay could be a noninvasive method for accurately and promptly diagnosing tuberculous serositis in patients in whom active tuberculosis in the cavity space is clinically suspected but for which no bacteriological evidence can be obtained.

Acknowledgments

We thank Yurika Ito, Ayako Watanabe, and Yasuko Inoue, for their technical assistance with the laboratory assays, and Drs. Kazuhiro Uchiyama, Toshiki Tamura, and Hajime Goto, for their support.

Financial support. The Ministry of Health, Labor and Welfare of Japan.

Potential conflicts of interest. All authors: no conflicts.

References

1. Dye C. Global epidemiology of tuberculosis. Lancet **2006**; 367:938–40.
2. Gopi A, Madhavan SM, Sharma SK, Sahn SA. Diagnosis and treatment of tuberculous pleural effusion in 2006. Chest **2007**; 131:880–9.
3. Ribera E, Ocana I, Martinez-Vazquez JM, Rossell M, Espanol T, Ruibal A. High level of interferon gamma in tuberculous pleural effusion. Chest **1988**; 93:308–11.
4. Ocana I, Martinez-Vazquez JM, Segura RM, Fernandez-De-Sevilla T, Capdevila JA. Adenosine deaminase in pleural fluids: test for diagnosis of tuberculous pleural effusion. Chest **1983**; 84:51–3.
5. Greco S, Girardi E, Masciangelo R, Capoccetta GB, Saltini C. Adenosine deaminase and interferon gamma measurements for the diagnosis of tuberculous pleurisy: a meta-analysis. Int J Tuberc Lung Dis **2003**; 7: 777–86.
6. Riantawan P, Chaowalit P, Wongsangiem M, Rojanaraweewong P. Diagnostic value of pleural fluid adenosine deaminase in tuberculous pleuritis with reference to HIV coinfection and a Bayesian analysis. Chest **1999**; 116:97–103.
7. Villena V, Lopez-Encuentra A, Echave-Sustaeta J, Martin-Escribano P, Ortuno-de-Solo B, Estenoz-Alfaro J. Interferon-gamma in 388 immunocompromised and immunocompetent patients for diagnosing pleural tuberculosis. Eur Respir J **1996**; 9:2635–9.
8. Burgess LJ, Maritz FJ, Le Roux I, Taljaard JJ. Combined use of pleural adenosine deaminase with lymphocyte/neutrophil ratio: increased specificity for the diagnosis of tuberculous pleuritis. Chest **1996**; 109:414–9.
9. Ocana I, Ribera E, Martinez-Vazquez JM, et al. Adenosine deaminase activity in rheumatoid pleural effusion. Ann Rheum Dis **1988**; 47: 394–7.
10. Laniado-Laborin R. Adenosine deaminase in the diagnosis of tuberculous pleural effusion: is it really an ideal test? A word of caution. Chest **2005**; 127:417–8.

11. Gupta UA, Chhabra SK. Diagnosing tubercular pleural effusions. Chest **2005**; 127:1078; author reply 1078–9.
12. Barnes PF, Mistry SD, Cooper CL, Pirmez C, Rea TH, Modlin RL. Compartmentalization of a $CD4^+$ T lymphocyte subpopulation in tuberculous pleuritis. J Immunol **1989**; 142:1114–9.
13. Stead WW, Eichenholz A, Stauss HK. Operative and pathologic findings in twenty-four patients with syndrome of idiopathic pleurisy with effusion, presumably tuberculous. Am Rev Tuberc **1955**; 71:473–502.
14. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet **2000**; 356:1099–104.
15. 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**; 7:155–60.
16. Arend SM, Andersen P, van Meijgaarden KE, et al. 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**; 181:1850–4.
17. 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**; 183:175–6.
18. Arend SM, Engelhard AC, Groot G, et al. 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**; 8:1089–96.
19. Mori T, Sakatani M, Yamagishi F, et al. Specific detection of tuberculosis infection: an interferon-gamma–based assay using new antigens. Am J Respir Crit Care Med **2004**; 170:59–64.
20. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics **1988**; 44:837–45.
21. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation **2007**; 115:928–35.
22. Wilkinson KA, Wilkinson RJ, Pathan A, et al. Ex vivo characterization of early secretory antigenic target 6-specific T cells at sites of active disease in pleural tuberculosis. Clin Infect Dis **2005**; 40:184–7.
23. Villena V, Lopez-Encuentra A, Pozo F, et al. Interferon gamma levels in pleural fluid for the diagnosis of tuberculosis. Am J Med **2003**; 115: 365–70.
24. Barnes PF, Fong SJ, Brennan PJ, Twomey PE, Mazumder A, Modlin RL. Local production of tumor necrosis factor and IFN-gamma in tuberculous pleuritis. J Immunol **1990**; 145:149–54.
25. Banales JL, Pineda PR, Fitzgerald JM, Rubio H, Selman M, Salazar-Lezama M. Adenosine deaminase in the diagnosis of tuberculous pleural effusions: a report of 218 patients and review of the literature. Chest **1991**; 99:355–7.
26. Burgess LJ, Maritz FJ, Le Roux I, Taljaard JJ. Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax **1995**; 50:672–4.
27. Valdes L, San Jose E, Alvarez D, Valle JM. Adenosine deaminase (ADA) isoenzyme analysis in pleural effusions: diagnostic role, and relevance to the origin of increased ADA in tuberculous pleurisy. Eur Respir J **1996**; 9:747–51.
28. Goto M, Noguchi Y, Koyama H, Hira K, Shimbo T, Fukui T. Diagnostic value of adenosine deaminase in tuberculous pleural effusion: a meta-analysis. Ann Clin Biochem **2003**; 40:374–81.

Lecture

解　説

HIV合併結核の現状と展望*

永井英明**

Key Words：HIV, AIDS, tuberculosis, HAART

はじめに

日本の結核の罹患率は結核対策により減少し2005年の結核罹患率は10万対22.2となったが，欧米先進国の結核罹患率が10以下であることを思えば，日本は結核については中進国である．また，HIV感染者数は2006年には3年連続で1,000名を越え，増加傾向にある．このような状況の日本では，今後HIV感染症合併結核の症例が増加する可能性がある．日本では森ら[1]によると2001年12月末までに222例の結核を合併したHIV感染者が確認されており，年々増加している．当院でも両者合併例は1992年以来徐々に増加し[2]，2006年末までに61例を経験している．

結核患者におけるHIV感染症の合併頻度については，全国レベルの調査はないが当院の症例について調べたところ[3]，抗HIV抗体陽性率は結核患者全体では3.2%，HIV感染症が疑われなかった症例では1.0%，粟粒結核では28.6%と高率であった．しかし，このデータは結核患者もHIV感染者も多い東京地区のデータであることを認識しておかなければならない．

今後もHIV感染症に合併した結核の増加が予想されるので，臨床の現場では注意が必要である．

HIV感染症における結核発病のリスク

HIVは主にCD4陽性Tリンパ球(CD4)に感染し，その細胞数が極端に減少することにより重篤な細胞性免疫障害が生じる．CD4の障害はさらにマクロファージ機能(抗原提示能，遊走能，活性化)の障害をもたらす．結核の感染防御にもっとも重要な働きを示すのは，CD4とマクロファージである．したがって，これらの細胞の機能障害が生じるHIV感染症では結核に感染し発病しやすい．細胞性免疫が低下した状態で結核を発病すると，肉芽腫の形成不全，結核菌の抑制不全，大量の結核菌による頻回の再燃，局所リンパ節への波及(肺門，縦隔リンパ節)，血行性の全身播種が起こりうる．この場合，乾酪性壊死と空洞形成は起こりにくくなる．

非HIV感染者が結核に感染した場合，結核が発病する確率は一生涯に5～10%といわれているが，HIV感染者が結核に感染した場合は，その発病する率は年間5～10%といわれ高率であり，その50%は2年以内に発病するといわれている[4]．

臨床像

結核菌は，HIV感染症に合併する日和見感染症をひき起こす病原体の中では比較的強毒性のため，結核症は早期(CD4数300～400/mm^3)から合併しやすい．症状は，発熱，倦怠感，体重減少，盗汗，咳嗽，喀痰などで，非HIV感染者の結核と同様であるが，他の日和見感染症にもみられる症状である．進行が速い場合があるので早期発見が重要である．ツベルクリン反応(ツ反応)は細胞性免疫の低下のため陰性であることが多い．

胸部X線写真では，免疫能が比較的保たれている時期では肺尖部に空洞形成を伴う典型的な

* Present state and outlook of HIV associated tuberculosis.

** Hideaki NAGAI, M.D.: 独立行政法人国立病院機構東京病院呼吸器科〔〒204-8585 清瀬市竹丘3-1-1〕; Department of Respiratory Medicine, National Hospital Organization Tokyo National Hospital, Kiyose 204-8585, JAPAN

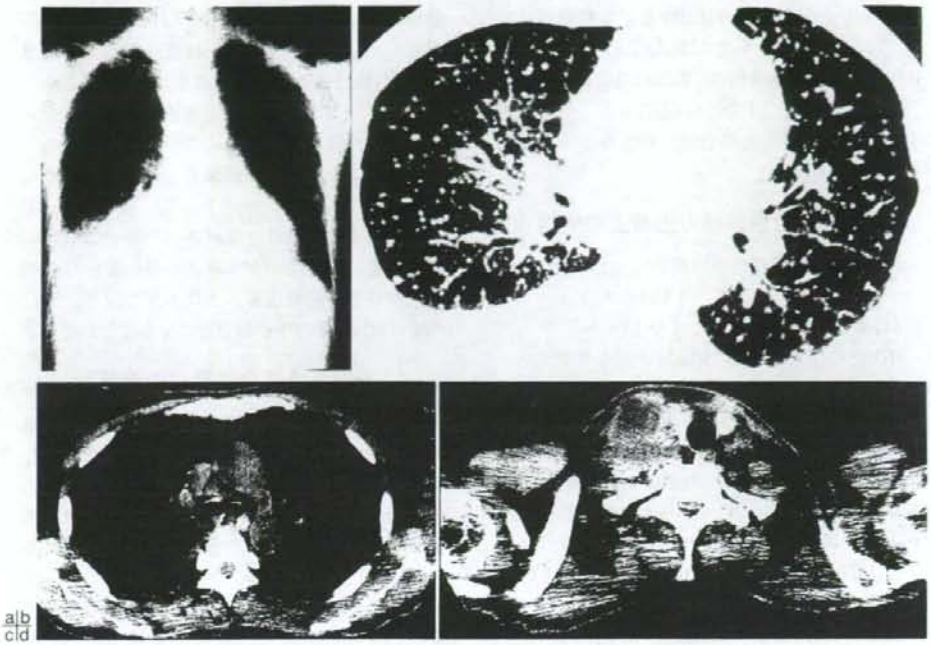

図1　40歳代のAIDS合併粟粒結核患者のX線写真
胸部単純X線写真(a)では右胸水，両肺びまん性の粒状影，傍気管リンパ節の腫大を認めた．胸部単純CT(b)，(c)では右胸水，粟粒影，多発小結節影，縦隔リンパ節の腫大を認めた．頸部単純CT(d)では頸部リンパ節の腫大を認め，内部は壊死様であり，腫大した縦隔リンパ節への連続性が認められた．

像を呈する．しかし，免疫能が低下した時期では，下葉の病変，非空洞形成，肺門・縦隔のリンパ節腫脹，粟粒影など非典型像を認めるようになる(図1)．

HIV感染症に合併した結核では，肺外結核の頻度が高いのが特徴である．肺外結核としては，リンパ節結核および播種型がもっとも多い．ほかに消化管，泌尿生殖器，中枢神経系の結核もしばしばみられる．HIV感染者では非HIV感染者に比較し，2倍の頻度で肺外結核を合併するといわれている．また，HIV感染者の中でも，肺外結核を合併した症例は，合併しない症例よりCD4数が低値である．

診　断

結核の罹患率の高いわが国では，HIV感染者に胸部異常影を認めた場合は常に結核の合併を念頭に置き，結核菌の検索を行うべきである．血液培養での結核菌の検出は，非HIV感染者の結核では稀であるが，HIV感染者の結核ではしばしば認められる．

強力な抗HIV療法(highly active antiretroviral therapy；HAART)時代におけるAIDSと結核

HAARTが導入されてからHIV感染症の予後は著明に改善し，AIDS関連疾患の減少とHIV感染者の死亡率の減少が認められている[5)~7)]．また，HAARTはHIV感染症における活動性結核の合併リスクを減少させたという報告もみられる[8)9)]．

HIV感染症合併結核症例に対してHAARTを行うことにより，予後の改善が得られたかという点についての報告は今のところ多くない．Girardiら[8)]によれば，HIV感染症合併結核症例に対して抗HIV療法を行わなかったかあるいは抗HIV薬を1剤しか投与しなかった群の死亡についてのhazard

ratioを1とした場合，抗HIV薬を3剤投与した群では0.14であり，有効なHAARTを行った場合，HIV感染症合併結核例の生存率は著明に改善したという．しかし，すでにHAARTを行っていた患者に結核を合併した場合は，むしろ予後不良であったという．

HIV感染症合併結核の治療上の問題点

HIV感染症合併結核の治療を行う上で注意すべき点としては，主に以下の3点がある．

(1)薬剤の副反応が起こりやすい．

HIV感染症では薬剤の副反応が起こりやすく，細心の注意を払う必要がある．とくに，抗結核薬では皮疹と肝障害の副作用が多い．抗結核薬と抗HIV薬を同時に内服する場合は両者の副反応を生じる可能性が高く，原因薬剤の同定が困難となるだけでなく，すべての治療を中断せざるをえない状況に追い込まれることがある．

(2)Rifamycin系薬剤と抗HIV薬との間に薬剤相互作用がある．

Rifamycin系薬剤〔rifampicin(RFP), rifabutin, rifapentine〕は肝臓と腸管においてcytochrome P450(とくにCYP3A4)の誘導作用が強い．CYP3A4により代謝されるプロテアーゼ阻害薬や非核酸系逆転写酵素阻害薬の血中濃度は，rifamycin系薬剤と併用することにより著しく低下し，抗HIV作用は低下する．また，逆にプロテアーゼ阻害薬は強力なCYP3A4抑制作用をもつ．このような理由から，プロテアーゼ阻害薬および非核酸系逆転写酵素阻害薬とRFPとの併用は注意が必要である．

結核の治療中に上記2系統の抗HIV薬を開始する場合は，RFPよりもCYP3A4の誘導が弱いrifabutin〔本邦では承認されておらず，エイズ治療薬研究班(東京医大臨床病理科)より譲り受ける〕を用いることが多かったが，CDC[10]はRFPとritonavir, ritonavir＋saquinavir, efavirenzとの併用を可能としたため選択肢が増えた．当院ではRFPとefavirenzの併用を行っているが，efavirenzの血中濃度の測定を行い，有効性を確認している．

(3)免疫再構築症候群が起こることがある．

結核治療中に早期にHAARTを開始した場合，結核の一時的悪化をみることがある[11]．症状・所見としては高熱，リンパ節腫脹，胸部X線所見の悪化(肺野病変および胸水の増悪)などがみられる．この反応は細胞性免疫能が回復し，生体側の反応が強くなったためにひき起こされると考えられており，免疫再構築症候群といわれている．

免疫再構築症候群と診断された場合は抗結核薬の変更は必要ないが，症状が強い場合は抗炎症剤や短期の副腎皮質ステロイドの投与，重症例では抗HIV薬の中止が必要になることがある．

HIV感染症合併結核の治療

感受性菌であれば，非HIV感染者における結核と同様に抗結核薬によく反応する．治療法としては，isoniazid(INH), RFP, pyrazinamide(PZA), ethambutol(EB)(あるいはstreptomycin)の4剤を2か月間投与し，その後INH, RFPの2剤(あるいはEBを加えた3剤)を4か月継続して，全治療期間を6か月とする，いわゆる短期療法でよいとされている[12]．しかし，最近rifamycin系薬剤による6か月治療では再発率が高く，治療期間を延長した方がよいという報告があり[13]，適切な治療期間について検討が必要であると指摘している．臨床的に効果の遅い症例や3か月以上結核菌の喀痰培養が陽性の症例では治療期間を延長すべきである．

多剤耐性菌の場合は予後不良であるが，感受性の残った薬剤とニューキノロン製剤などを用い，長期の治療が必要となる．

HAARTの開始時期

結核の診断がついたときにすでに以前よりHAARTを行っている患者では，HAARTが有効であれば抗HIV薬はそのまま継続し，結核の治療を開始する．HAARTが有効でなければ中止し，結核の治療が2か月終了した時点で新たなHAARTを開始する．

結核の診断がついた時点で抗HIV薬の投与を行っていない症例については，結核の治療を優先する．結核の治療を失敗した場合，死に至る可能性があるためだけでなく，周囲への二次感染をひき起こし，多剤耐性結核菌の出現をもたらす

表1　エイズ結核合併例にHAARTをいつから開始するべきか？

WHO 2004[14]	BHIVA 2005[15]	Johns Hopkins University 2006[16]	DHHS 2005[17] ATS, CDC, IDSA 2003[18]
CD4数＜200/mm³	＜100/mm³	＜50/mm³	
結核の治療を開始し，結核の治療に耐えられることがわかれば，早期に(2週間から2か月の間)開始			HAARTと結核の治療を同時に始めてはいけない．4～8週間遅らせてHAARTを開始すること(CD4数＜50のときは例外的に同時に始める場合がある)．
200～350	100～200	50～200	
結核の導入療法終了(2か月後)以降に開始を考慮			
＞350	＞200	200～350	
結核の治療を開始し，結核の治療終了後に開始			

可能性があるからである．

結核の治療開始後に新たにHAARTを開始する場合は，「HIV感染症合併結核の治療上の問題点」の項で示した3点についての配慮が必要であり，いつからHAARTを開始すべきか悩む症例が多い．HAARTの開始時期について，evidenceの明確な推奨はない．

HAARTの開始時期はCD4数により以下の3つに分けられる．

(1)CD4数がきわめて少ないためにできるだけ早急に始める．

(2)CD4数に余裕があり，2か月間，結核の治療を行い，その後HAARTを開始する．2か月後ではPZAが終了し薬剤数が減り，さらに免疫再構築症候群が起こりにくくなり，HAARTを開始しやすくなる．

(3)CD4数が多いので結核の治療が終了してからHAARTを開始する．

この3パターンを分けるCD4数の基準が，表1のように種々のガイドラインで異なり[14)～18)]，HAARTの開始時期に苦慮する．

筆者はできるだけHAARTを遅らせるという方針をとっている．CD4数が200/mm³以下であれば4～8週後(待てるのであれば8週後)，200～350/mm³は2か月以後で症例ごとに考慮，350/mm³以上であれば結核の治療終了後，としている．ところが，抗結核薬の副作用やその他の合併症のために，予定通りHAARTを開始できない例が多いのが実情であり，症例ごとの配慮が必要である．

結核の予防

米国ではHIV感染者に対してはツ反応を行い，硬結が5 mm以上を陽性とし，結核の感染ありとした．これらの患者では活動性結核を合併しているかどうかの精査を行い，活動性結核がない場合はINHの予防投与(300mg/日，9か月間)を行うとしている[18)]．本邦ではBCGの施行例も多く，HIV感染者におけるツ反応の評価は難しい．

しかし，最近，結核感染診断法としてリンパ球のインターフェロン-γ産生能を測定する方法(QFT-2G)が開発され，HIV感染症合併結核例での有用性が示唆されている[19)]．免疫機能が著しく低下した症例では陽性コントロールにも反応しなくなり判定不可例が出る可能性があるが，当院のエイズ合併結核13例では，判定不可例は1例，陽性率76.9%とツ反応の15.4%に比較し，感度は有意に高かった．

HIV感染者では，BCGによる予防は禁忌である．播種性の*M.bovis*症を合併した症例があるからである．

文　献

1) 森　亨，中田　光，永井英明，ほか．HIV陽性抗酸菌症の動向と診療のあり方に関する研究．In：厚生科学研究費補助金エイズ対策研究事業『日和見感染症の治療に関する研究』平成14年度研究報告書．2003. p. 24.

2) 永井英明，蛇沢　晶，赤川志のぶ，ほか．Human Immunodeficiency Virus(HIV)感染症における結核．日胸疾会誌 1997；35：267.

3) 永井英明，川辺芳子，長山直弘，ほか．結核患者における抗HIV抗体陽性率の検討．結核 2001 ; 76 : 679.
4) Daley CL, Small PM, Schecter GF, et al. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus. N Engl J Med 1992 ; 326 : 231.
5) Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced HIV infection. N Engl J Med 1998 ; 338 : 853.
6) Mouton Y, Alfandari S, Valette M, et al. Impact of protease inhibitors on AIDS-defining events and hospitalizations in 10 French AIDS reference centres. Federation National des Centres de Lutte contre le Sida. AIDS 1997 ; 11 : F101.
7) Ledergerber B, Egger M, Erard V, et al. AIDS-related opportunistic illnesses occurring after initiation of potent antiretroviral therapy. JAMA 1999 ; 282 : 2220.
8) Girardi E, Palmieri F, Cingolani A, et al. Changing clinical presentation and survival in HIV-associated tuberculosis after highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2001 ; 26 : 326.
9) Kirk O, Gatell JM, Mocroft A, et al. Infections with Mycobacterium tuberculosis and Mycobacterium avium among HIV-infected patients after the introduction of highly active antiretroviral therapy. Am J Respir Crit Care Med 2000 ; 162 : 865.
10) Centers for disease control and prevention. Updated guidelines for the use of rifabutin or rifampin for the treatment and prevention of tuberculosis among HIV-infected patients taking protease inhibitors or nonnucleoside reverse transcriptase inhibitors. MMWR 2000 ; 49 : 185.
11) Narita M, Ashkin D, Hollender ES, et al. Paradoxical worsening of tuberculosis following antiretroviral therapy in patients with AIDS. Am J Respir Crit Care Med 1998 ; 158 : 157.
12) Centers for disease control and prevention. Prevention and treatment of tuberculosis among patients infected with human immunodeficiency virus : Principles of therapy and revised recommendations. MMWR 1998 ; 47(RR-20) : 1.
13) Nahid P, Gonzalez LC, Rudoy I, et al. Treatment outcomes of patients with HIV and tuberculosis. Am J Respir Crit Care Med 2007 Feb 8 [published ahead of print].
14) World Health Organization. Scaling up antiretroviral therapy in resource-limited settings : treatment guidelines for a public health approach. 2003 revision. Geneva : World Health Organization ; 2004. p. 40.
15) Pozniak AL, Miller RF, Lipman MCI, et al. BHIVA treatment guidelines for TB/HIV infection. 2005 February p. 16. Available from : URL : http://www.bhiva.org.
16) Bartlett JG. Pocket Guide to Adult HIV/AIDS Treatment. Johns Hopkins HIV Care Program 2006 p. 69. Available from : URL : http://www.hopkins-aids.edu/.
17) Department of Health and Human Services(DHHS). Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents 2005. p. 37.
18) American Thoracic Society, CDC, and Infectious Diseases Society of America. Treatment of Tuberculosis. Am J Respir Crit Care Med 2003 ; 167 : 603.
19) 永井英明，有賀晴之，川辺芳子，ほか．AIDS合併結核におけるQuantiFERON-TB第2世代の有用性についての検討．結核 2005 ; 80 : 334.

* * *