症例報告

多彩な胸部異常陰影を呈し，経気管支鏡生検材料でのDNA診断が有用であった肺結核の1例

阿部　良行　藤野　忠彦　橋詰　寿律　鈴木　国功
菊池　敬一

国立療養所神奈川病院呼吸器科

A CASE OF PULMONARY TUBERCULOSIS DIAGNOSED BY DNA AMPLIFICATION METHODS FROM TRANSBRONCHIAL LUNG BIOPSY MATERIALS

*Yoshiyuki ABE, Tadahiko FUJINO, Toshinori HASHIZUME, Kuninori SUZUKI, and Keiichi KIKUCHI

*Department of Respiratory Disease, National Sanatorium Kanagawa Hospital

The hybridization assay using polymerase chain reaction (PCR) is useful for rapid detection of *Mycobacterium tuberculosis* (*M. tuberculosis*). A 77-year-old female was admitted to our hospital complaining of cough, and examination of the sputum culture showed *M. tuberculosis*. Her chest X-ray showed a variety of abnormal shadows, such as a cavity lesion, multiple coin lesions, and infiltrates. Malignant disease was also suspected to be involved, with the complication of pulmonary tuberculosis. Specimens were obtained by transbronchial lung biopsy (TBLB) from coin lesions. The hybridization assay using PCR on the TBLB specimens showed *M. tuberculosis* gene expression. She was treated with anti-tuberculous drugs. All shadows in her chest X-ray were improved six months after admission. She was remained well without recurrence for more than two years after admission. The hybridization assay using PCR with TBLB specimens is useful for the detection of *M. tuberculosis*.

症例は77歳の女性で，胸部X線写真にて空洞性病変と多発性円形陰影および，両側下肺野に網状影を認めた。喀痰検査にて，塗抹は陰性であったが，PCR法にて結核菌陽性であった。多発性円形陰影は，転移性肺腫瘍も考えられたため，気管支鏡検査を施行し，右S^8の腫瘤の生検（TBLB）を行った。組織学的には肉芽腫性病変であったが，Ziehl-Neelsen染色は陰性であった。TBLB切片を用いたPCR法にてこの病変も結核と診断した。抗結核薬の投与により，喀痰の培養は陰性化し，多発性円形陰影を含めた胸部異常陰影も改善した。

*〒259-8585 神奈川県秦野市落合666-1

*666-1, Ochiai, Hadano-shi, Kanagawa 259-8585 Japan.
(Received 17 Jul. 2000／Accepted 19 Sep. 2000)

Key words : Pulmonary tuberculosis, Polymerase chain reaction (PCR), Transbronchial lung biopsy (TBLB), DNA

キーワーズ : 肺結核, PCR 法, 経気管支鏡生検, DNA

Introduction

Tuberculosis is one of typical re-emerging diseases, affecting patients in both developing and industrialized countries[1]. Cavities in the lungs are the most common findings in human tuberculosis. From a cavity, the bacilli enter the bronchial tree and spread to other parts of the lung and also to other people. Radiological findings in pulmonary tuberculosis vary, and include cavities, coin lesions, infiltrates, miliary shadows, and small or large nodules. Chest X-rays are very important because the character of the shadows indicates the source of the disease and computed tomography contributes to the diagnosis of pulmonary tuberculosis by providing details[2,3].

The hybridization assay using polymerase chain reaction (PCR) is useful for rapid and specific detection of *Mycobacterium tuberculosis* (*M. tuberculosis*), and usually this assay is performed with specimens of sputum, gastric juice, bronchioalveolar lavages fluid (BALF) or pleural fluid[4]. However, the location of the lesions in this case was not suitable for the above specimens. Fusegawa et al. reported that biopsy specimens could also be used for detection of *M. tuberculosis* by the hybridization assay with PCR[5].

We present a case of pulmonary tuberculosis showing a variety of shadows on the chest X-ray, among which multiple coin lesions suggested a metastatic lung tumor. We diagnosed pulmonary tuberculosis from specimens of transbronchial lung biopsy (TBLB) of the coin lesions by PCR assay. Treatment with antituberculous drugs resolved the clinical and radiographic abnormalities, and the patient remains in good health two years and eight months after admission.

Case report

A 77-year-old female was admitted to our hospital complaining of cough on January 28th, 1998, and examination of the sputum culture showed *Mycobacterium tuberculosis* (*M. tuberculosis*) without an acid-fast organism in her sputum smear. A physical examination revealed the following: pulse 102 and regular, blood pressure 120/70 mmHg, respiratory rate 23, and temperature 35.4℃. The head, eyes, ears, nose and throat examinations were unremarkable. There was no adenopathy. An examination of the lungs revealed fine crackles in both lower fields. The cardiac sounds were regular, and no murmur was noted. There was no clubbing, cyanosis, or edema.

Initial laboratory results included a WBC of 6.090 mm^3 with 62.2 neutrophils, 22.7 lymphocytes, 9.3 monocytes, 3.4 eosinophils, and 0.3 basophils, hemoglobin at 13.6 g/d*l*, and a hematocrit of 42.0%. Biochemical data including total protein were within normal limits. Serum hemoglobin A1C level was elevated to 11.5% (normal, 2.5-6.0%), and urinalysis showed glucose (2+). The patient was thus diagnosed with diabetes mellitus. Arterial blood gas measurements showed a pH of 7.412, Po2 of 55.5 torr, and a Pco2 of 39.0 torr while breathing room air. While the examination of sputum smear was negative, sputum culture showed 1 colony of *M. tuberculosis* at 8 weeks. The hybridization assay using PCR with sputum was positive. The values of carcinoembryonic antigen and SCC were within normal limits (1.5 ng/m*l* and 1.3 ng/m*l*, respectively). Her chest X-ray showed a cavity lesion in the right upper field (5.0×4.0 cm), multiple nodular lesions in bilateral lung fields (up to 2 cm), and infiltrates in both lower fields (Fig. 1). Chest tomography (Fig. 2a) and CT scan (Fig. 3) also showed the above shadows.

Malignant disease was also suspected to be involved, with the complication of pulmonary tuberculosis. Bronchoscopy was performed on February 4th. The specimens were obtained by transbronchial lung biopsy (TBLB) from nodular lesions of right S^8. TBLB specimens were processed for routine histopathological analysis and also for DNA analysis by PCR. The hybridization assay using PCR showed *M. tuberculosis* in the TBLB specimens.

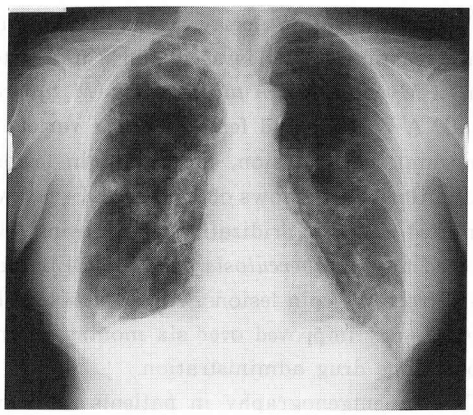
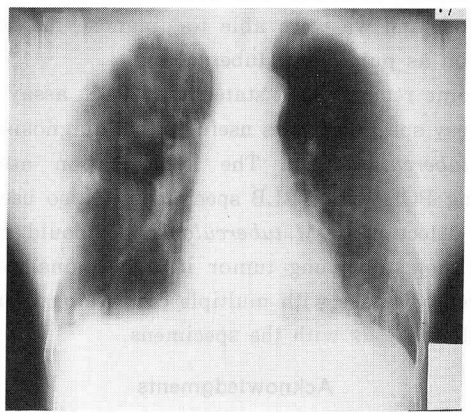


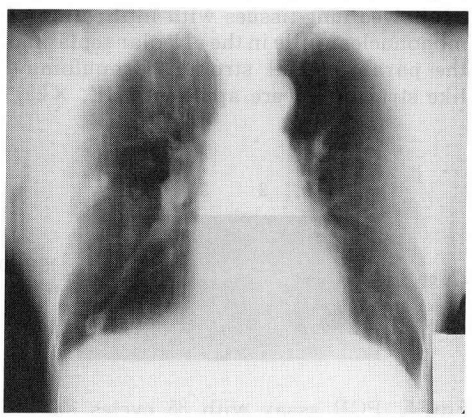
Fig. 1 Chest X-ray on admission showed a cavity lesion in the right upper field (5.0×4.0 cm), multiple nodular lesions in bilateral lung fields (up to 2 cm), and infiltrates in both lower fields.

From January 31st, the patient was administered antituberculous drugs (isoniazid ; INH, 0.3 g : rifampicin ; RFP, 0.45g : ethambutol hydrochloride ; EB, 0.75g). *In vitro* drug sensitivity testing showed sensitivity to almost all drugs including INH, RFP, and EB. On June 14th, she was discharged from our hospital because the culture of sputum showed no *M. tuberculosis*, and she was subsequently followed in our outpatient clinic with administration of INH, EB and RFP. Just prior to discharge from the hospital, her serum hemoglobin A1C level was 9.0%. Arterial blood gas measurements showed a pH of 7.403, Po2 of 63.7 torr, and Pco2 of 39.1 torr while breathing room air. All shadows including the nodular shadows in her chest X-ray had improved six months after admission (Fig. 2b). She remains well without recurrence more than two years after admission.

We performed PCR assay based on co-amplification of the insertion sequence of IS6110 gene that is species-specific for *M. tuberculosis* complex, according to our previous report[5)6)]. DNA was prepared from TBLB specimens (about 1 mm^3) with ethanol preparation by routine procedures[7)8)]. The following primers were used for IS6110 : sense primer ISF-1 : 5′-TGCGCGATGG CGAACTCAAGG-3′, antisense primer ISF-2 : 5′-GTCTGCTACCCACAGCCGGTT-3′. PCR



(a)



(b)

Fig. 2 Chest tomography showed multiple coin lesions on admission (a) which were improved on discharge from hospital (b).

products were observed under ultraviolet light.

The sections of TBLB from right S^8 showed lung tissue with infiltration of mononuclear cells in the alveolar septa and the parabronchial stroma. Granuloma-like structures were apparent (Fig. 4), but Ziehl-Neelsen staining failed to show microorganisms in the lung. No malignancy was seen. The above histopathological features were consistent with those of pulmonary tuberculosis.

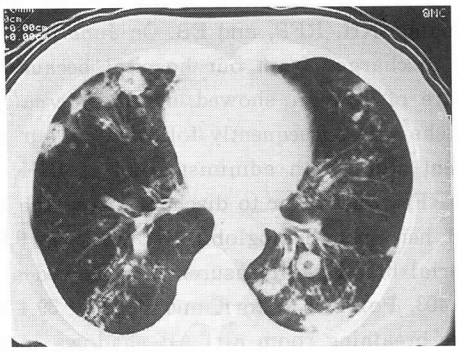


Fig. 3 Chest CT scan showed multiple coin lesions with small cavities in the both lung fields on admission.

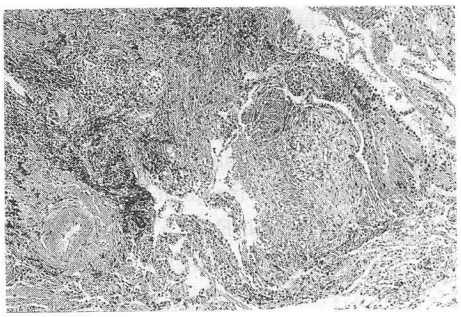


Fig. 4 The sections of TBLB from right S^8 showed lung tissues with infiltration of mononuclear cells in the alveolar septa and the parabronchial stroma. Granuloma-like structures were apparent (HE, ×25).

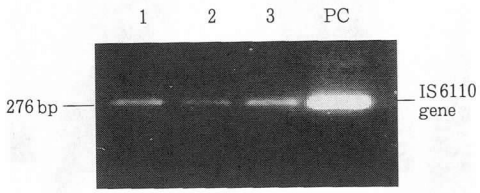


Fig. 5 PCR assay with 35 cycles showed a specific band for *M. tuberculosis* in the specimen of TBLB obtained from right S^8. PC ; positive control, 1-3 ; TBLB specimens of this case.

PCR assay showed a specific band for *M. tuberculosis* in the specimen of TBLB obtained from right S^8 (Fig. 5).

Discussion

The hybridization assay using polymerase chain reaction (PCR) is useful for rapid detection of *Mycobacterium tuberculosis* (*M. tuberculosis*). A 77-year-old female with a variety of abnormal (cavity lesion, multiple coin lesions, and infiltrates) shadows on her chest X-ray was admitted and a hybridization assay using PCR showed the *M. tuberculosis* gene in TBLB specimens from the coin lesions. All shadows on her chest X-ray improved over six months of antituberculous drug administration.

Chest roentgenography in patients with pulmonary tuberculosis can show many findings[9], and CT scan can also provide important information regarding active pulmonary tuberculosis[2)3)]. Multiple coin lesions on a chest X-ray are suggestive of metastatic lung cancer. Therefore, we performed the PCR assay not with specimens of BALF but with TBLB from coin lesion, and we were able to diagnose the coin lesion as pulmonary tuberculosis.

Some reports have stated that PCR assay of biopsy specimens was useful in the diagnosis of *M. tuberculosis*[5)7)8)]. The hybridization assay using PCR with TBLB specimens is also useful for detection of *M. tuberculosis*. We could rule out metastatic lung tumor in a pulmonary tuberculosis case with multiple coin lesions using PCR methods with the specimens.

Acknowledgments

We thank Dr. Hisae Fusegawa (Department of Clinical Pathology, Tokai University) for technical assistance of TB-PCR analysis. We also thank Dr. Masato Nakamura and Dr. Masami

Yasuda (Department of Pathology, Tokai University) for excellent suggestions regarding pathological diagnosis.

References

1) Raviglione MC, Snider Jr DE, Kochi A: Global epidemiology of tuberculosis: morbidity and mortality of a worldwide epidemic. JAMA. 1995; 273: 220−226.
2) Im J, Itoh H, Shim Y, et al.: Pulmonary tuberculosis: CT findings—early active disease and sequential change with antituberculous therapy. Radiology. 1993; 186: 653−660.
3) Primack SL, Logan PM, Hartman TE, et al.: Pulmonary tuberculosis and mycobacterium avium-intracellulare; a comparison of CT findings. Radiology. 1995; 194: 413−417.
4) Fusegawa H, Miyachi H, Ohshima T, et al.: Detection of mycobacterium tuberculosis by amplification of mycobacterial DNA from pleural fluid and gastric juice: The comparison of IS6110 gene with Amplicor™ mycobacterium. Jpn J Clin Pathol. 1995; 43: 941−947.
5) Fusegawa H, Miyachi H, Ohshima T, et al.: Rapid detection and identification of mycobacteria by the PCR assay based on the co-amplification of the gene IS6110 and groEL. Jpn J Clin Pathol. 1995; 43: 1051−1056.
6) Miyachi H, Fusegawa H, Ohshima T, et al.: Dihydrofolate reductase gene expression characterized by the PCR assay in human leukemia cells. In Vivo. 1991; 5: 7−12.
7) Ohta M, Tokuda Y, Yanagita Y, et al.: A case of tuberculous cervical tumor diagnosed by polymerase chain reaction assay. Jap J Clin Surg. 1993; 54: 1497−1501.
8) Ueno S, Fusegawa H, Yokoyama S, et al.: A case of multiple tuberculoma diagnosed by polymerase chain reaction assay. Jap J Surg. 1993; 95: 200−203.
9) Fraser RG: Mycobacterial infection of the lungs. In: Diagnosis of disease of the chest, 4th Eds Fraser RG, Pare JAP, Pare PD, et al. WB. Saunders Company, Philadelphia, 1995, 910−923.