

Original Article

A CLINICAL STUDY ON CASES WITH PULMONARY *M. AVIUM* COMPLEX (MAC) DISEASE FOLLOWED UP FOR MORE THAN 10 YEARS

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Abstract We performed a clinical study on 22 cases with pulmonary *M. avium* complex disease followed up for more than 10 years. The mean age of these patients at the first visit was 60.1 years old and the extent of the disease among them was minimum or moderate. The progression of the disease was relatively slow. There were 5 cases of tuberculosis-like type and 11 cases of nodular-bronchiectasis type among the primary infection type and 6 cases of the secondary infection type. Classifying these cases by the grade of progression on chest X-ray findings, 2 cases improved, 11 cases slightly progressed, 4 cases moderately progressed and 5 cases severely progressed. Seven cases of the nodular-bronchiectasis type of the primary infection type were observed among 11 slightly progressed cases. This suggested that the prognosis of cases with nodular-bronchiectasis type was relatively good. The frequency of a reagravation of chest X-ray findings was significantly higher in the severely progressed cases than in the slightly and moderately progressed cases. Generally, the number of used drugs was more and the duration of treatment was longer in the severely progressed cases. This suggested

that these cases were intractable. The results of sputum culture for *M. avium* complex were consistent with the progression of the disease shown on chest X-ray findings among many cases. However, sputum culture negative was observed in some cases with the nodular-bronchiectasis type in spite of the reagravation on chest X-ray findings. On the other hand, there were some cases in which few reagravation was observed in spite of continued sputum culture positive. Based on these results, we made some remarks on the treatment and management of pulmonary *M. avium* complex disease.

Key words: *M. avium* complex, Long-term follow up cases, Chemotherapy, Prognosis

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Original Article

A CLINICAL STUDY ON TUBERCULOSIS AMONG YOUNG ADULTS IN JAPAN:
ANALYSIS ON PATIENTS ADMITTED TO NATIONAL HOSPITALS
IN KANTO- AND KINKI-AREAS IN THE YEAR 2000

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Abstract Considering the high social activity, the trend of tuberculosis among young adults appears to be one of the key factors that influence the future morbidity rate of tuberculosis in Japan. To investigate its current characteristics, we analyzed new cases of tuberculosis aged 20 to 29 who were admitted to 7 national hospitals in Kanto- and Kinki-areas during the period of January 1st to December 31st, 2000. Data on the following items were compiled : sex, age, body height and weight, nationality ; background factors such as life style, complications ; course of the disease before the diagnosis ; result of PPD skin test ; severity of the disease estimated by the amount of *M. tuberculosis* in sputum and the grade of chest X-ray findings ; therapeutic regimens and the response rate. Data were collected from 234 patients (129 males and 105 females) and the results were as follows: 1) about 80% of the patients were symptomatic and in 50% of patients who presented with cough, more than one month was needed before establishing the diagnosis as TB, 2) the disease was found in advanced stage in more than half of the patients, 3) foreigner patients, most of them were from Kanto-area, accounted for 11%, and were in advanced stage, some with drug-resistant tuberculosis, 4) INH resistance was noted in

7.7%, 5) pyrazinamide was included in the therapeutic regimens in 84.0% of the smear positive patients, 6) the admission period was within 90 days in 63.7% of the patients, however, the duration of treatment was 6 months in only 48.0% of patients who were treated with regimens containing pyrazinamide. More efforts for early detection of patients is needed to prevent the transmission of the disease, and more extensive use of directly observed therapy is essential for the prevention of dropout. We also argued about the shortening of the admission and duration of treatment in these patients.

Key words: Young adult tuberculosis, Tuberculosis in foreigners

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Original Article

COMPARATIVE EVALUATION OF THE ISOTHERMAL AND CHIMERIC PRIMER-INITIATED AMPLIFICATION OF NUCLEIC ACIDS (ICAN[®]) AND ROCHE AMPLICOR[®] PCR AND CULTURE FOR DETECTING *MYCOBACTERIUM TUBERCULOSIS* COMPLEX IN SPUTUM SAMPLES

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Abstract We compared the ability of the newly developed ICAN[®] MTB Detection Kit (TaKaRa Bio Inc.), which uses the Isothermal and Chimeric primer-initiated Amplification of Nucleic acid (ICAN), with that of COBAS Amplicor PCR System (Roche Diagnostics) to directly detect *Mycobacterium tuberculosis* complex (MTB) in sputum samples.

A total of 142 sputum samples from 120 patients were examined in this study. The results were compared with those of acid-fast staining and MGIT liquid culture system (BD) following identification by the probe test (DDH Mycobacteria Kit). A total of 68 specimens were MGIT[®] positive for MTB. In addition, 62 specimens were positive by the combination of staining and MGIT assay for MTB. When compared with that for MGIT, the sensitivity of each assay system was 88.2% for ICAN and 92.6% for COBAS Amplicor, respectively. The specificity of each assay system was 65.7% for ICAN and 62.7% for COBAS Amplicor, respectively.

Coincidence between ICAN and COBAS Amplicor assay results was 96.3% (130 of 135 samples). No significant difference was observed between the results of the two assay methods.

It is concluded that although both nucleic acid amplification methods are sensitive and specific for the detection of MTB in the respiratory specimens, ICAN system appeared to be more rapid (within 3.5 h from the specimen collection) than Ampli-cor system. The ICAN system will be useful in clinical laboratories for the rapid detection of MTB without specially programmed thermo-cycler.

Key words: Nucleic Acid Amplification, *Mycobacterium tuberculosis* complex, Isothermal and Chimeric primer-initiated Amplification of Nucleic Acid, Acid-fast staining, Liquid culture

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The 78th Annual Meeting Special Lecture

STANDARDIZATION OF LABORATORY TESTS FOR TUBERCULOSIS
AND THEIR PROFICIENCY TESTING

Chiyoji ABE

Abstract Explanations of proper collection procedures are imperative for accurate laboratory analysis. The quality of specimens collected and the proper transport of those specimens to the laboratory are critical to successful isolation of etiological agents.

Most mycobacteria grow at a relatively slow rate. Therefore, the acid-fast smear plays an important role in the early diagnosis of mycobacterial infection. There are several methods of determining the acid-fast nature of an organism. In the carbolfuchsin procedures (Ziehl-Neelsen, Kinyoun), acid-fast organisms appear red, and in the fluorochrome procedures (auramine O, auramine-rhodamine, acridine orange), the acid-fast organisms fluoresce yellow to orange. Fluorochrome-stained slides may be directly restained with any of the carbolfuchsin staining procedures. This may be done to confirm a positive fluorochrome slide and to study organism morphology.

In the last 10 years, there were many advances in the culture examinations of mycobacteria. The newly developed Mycobacteria Growth Indicator Tube (MGIT), BacT/Alert, ESP Myco, MB Redox and KRD "Nichi B", biphasic Septi-Chek AFB and Myco-Acid, and radiometric BACTEC 460TB systems based on liquid media, proved to be significantly better than the egg-based solid media for the isolation of mycobacteria from clinical specimens. In addition to liquid-based medium, agar (Middlebrook 7H10 or 7H11) - or egg (Ogawa or Löwenstein-Jensen)-based media should be used in the primary isolation of mycobacteria.

To identify mycobacteria, conventional biochemical tests are traditionally used. Key test can be used to identify species, or further preliminary grouping may be used. Other approaches to identifying some species of mycobacteria are available. They include niacin accumulation, *p*-nitrobenzoic acid and *p*-nitro- α -acetyl-amino- β -hydroxypropionophenone tests for discrimination of the *Mycobacterium tuberculosis* complex from mycobacteria other than *M. tuberculosis* (MOTT); DNA probe methods for identification or confirmation of the *M. tuberculosis* complex, *M. avium* complex, *M. kansasii*, and *M. gordonae*; DNA-DNA hybridization

method for identification of 22 *Mycobacterium* species; and gas-liquid chromatography or high performance liquid chromatography analyses for recognizing the patterns of the mycobacterial cell wall fatty acids or mycolic acids. The advantages of the last four methods are that they are capable of providing definitive identification within 2 to 5 h after adequate growth. Capilia TB is the newly developed immunochromatographic assay for rapid discrimination between the *M. tuberculosis* complex and MOTT bacilli. The kit can be easily used for rapid identification of the *M. tuberculosis* complex in combination with the culture systems based on liquid media. In addition, Capilia TB could correctly detect the *M. tuberculosis* complex from mixed cultures with the *M. tuberculosis* complex and MOTT bacilli.

The WHO/IUATLD supranational reference laboratory (SRL) network was created in 1994, to ascertain the accuracy of the susceptibility test methods used in different laboratories across the world, and to allow comparability of the surveillance data gathered in countries participating in the Global Project on Anti-tuberculosis Drug Resistance Surveillance. Today, the network has evolved and 23 SRLs actively participate. Results of five rounds of proficiency testing in the SRL network suggest that performance of the network has improved substantially through the years. This progress has been particularly evident for streptomycin and ethambutol sensitivity, which was very low in the first rounds of proficiency testing. In 1998 sensitivity for these two drugs was higher than 95%. For isoniazid and rifampin, sensitivity has been consistently high since the beginning of the Global Project. This indeed reflects the enhanced efforts made by the SRLs to improve their individual performance.

Key words: Laboratory tests for tuberculosis, early diagnosis of mycobacterial infection, liquid medium, immunochromatographic assay, proficiency testing

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————— Memorial Lecture by the Imamura Award Winner —————

CLINICAL, RADIOLOGICAL, AND PATHOLOGICAL FINDINGS OF
NON-TUBERCULOUS MYCOBACTERIA RESPIRATORY INFECTION

Jiro FUJITA

Abstract This study is designed to evaluate radiological, clinical, and pathological findings of *Mycobacterium avium* complex (MAC) respiratory infection. Two-hundreds of non-tuberculous mycobacteria obtained from upper respiratory tract were collected. Among them, 88 cases were selected according to the strict diagnostic criteria of MAC and chest CT findings were evaluated in 67 cases. In addition, successive chest CT findings were evaluated in 25 cases with MAC respiratory infection. Furthermore, pathological findings were evaluated in 9 surgically-resected lung specimens. Fever, hemoptysis, and dyspnea were more frequently observed in smear-positive patients than in smear-negative, culture-positive patients. Centrilobular nodules and bronchiectasis are frequent observations in patients with MAC. In addition, cavity formation was more frequently observed in smear-positive patients compared with smear negative-cases. Since the score of bronchiectasis in the second CT was significantly higher than in the first CT, progression of bronchiectasis appeared to be caused by MAC infection. Pathologically, extensive granuloma formation throughout the airways was clearly demonstrated. Immunohistochemical staining demonstrated: 1) epithelioid cells and giant cells;

and 2) myofibroblasts extensively infiltrating the cavity wall. When granuloma was initially formed, no myofibroblasts were found, but as caseous necrosis appeared, the thin epithelioid cell layer was detected and the outer myofibroblast layer gradually became thick. In the cavity wall, the layer of epithelioid cells and multinucleated giant cells surrounded necrosis, and was associated with the outer layer of myofibroblasts. In addition, the anti-TGF-beta 1 antibody stained the cytoplasm of epithelioid cells and multinucleated giant cells, preceding the advent of myofibroblasts.

Key words: Non-tuberculous mycobacteria, *Mycobacterium avium* complex, clinical features, radiological findings, pathological findings

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RADIOLOGICAL FINDINGS OF NON-TUBERCULOUS MYCOBACTERIA RESPIRATORY INFECTION

Jiro FUJITA

Abstract In these 15 years, the clinical importance of non-tuberculous mycobacteria respiratory infection has been increasing. Especially, from the report of Prince et al., it has been suggested that *Mycobacterium avium* complex (MAC) respiratory infection is increasing especially in elderly women without underlying diseases. In MAC respiratory infection, right middle lobe and left lingula are frequently involved and centrilobular nodules and diffuse bronchiectasis are characteristic radiological findings. In addition, successive evaluation of chest CT has demonstrated that grade of bronchiectasis deteriorated, suggesting that bronchiectasis is caused by MAC respiratory infection. More recently, pathological findings of MAC respiratory infection as well as the pathogenesis of

MAC respiratory infection has been partially clarified.

Key words: Non-tuberculous mycobacteria, *Mycobacterium avium* complex, Radiological findings, Bronchofiberscope, Clinical symptom, Pathological findings

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