Original Article

COMPARATIVE EVALUATION OF ACID-FAST STAINING FOR THE DETECTION OF *MYCOBACTERIUM FORTUITUM*

— Clinical Performance of Fluorescent and Ziehl-Neelsen Staining —

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Abstract [Objective] Fluorescent staining is of paramount importance, not only for confirming the presence of mycobacteria in a given specimen but also for providing an estimated growth quantification. In this study, for rapidly growing *Mycobacterium fortuitum*, we evaluated the effectiveness of a rapid fluorescent staining method employing auramine-rhodamine (AR) fluorescent stain and acridine-orange (AO) fluorescent stain compared to that of the standard Ziehl-Neelsen (ZN) stain currently in use in our laboratory.

[Method] We evaluated the acid-fast nature of *M.fortuitum* strain ATCC6841 and 42 clinical isolates from each patient diagnosed at NHO Kinki-chuo Chest Medical Center. These isolates were preliminarily identified as *M.fortuitum* using DNA-DNA hybridization (DDH Mycobacteria; Kyokuto Pharmaceutical, Tokyo, Japan). These isolates were further identified by comparative sequence analysis of the ITS regions and the partial 16S rRNA gene.

[Results] A total of 26 *M.fortuitum* strains (61.9%) demonstrated the lack of an acid-fast nature by AR staining, and slightly fewer demonstrated the same by AO staining. Sequence analysis of these 42 clinical isolates led to the identification of 35 *M.fortuitum* subsp. *acetamidolyticum* isolates (83.3%) and 7 closely *M.fortuitum* isolates.

[Discussion] This work reported the loss of the acid-fast nature of specific *M.fortuitum* strains. It is likely that both the specific cell envelope of *M.fortuitum* and the staining mechanics could have been responsible for the loss of the acid-fast nature since the 2 different fluorescent stains yielded the same results. *M.fortuitum* is a mycobacterium species that does not stain with the commonly used fluorescence microscopy technique. Therefore, we suggested the use of an identification scheme for these organisms that employs ZN staining and the study of cultural characteristics (growth rate, temperature, and pigment production).

Key words: *Mycobacterium fortuitum*, Ziehl-Neelsen staining, Fluorescent staining, 16S−23S ITS sequencing

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Original Article

A STUDY OF RELAPSE/RECURRENCE CASES AFTER SURGICAL TREATMENT FOR PATIENTS WITH PULMONARY NONTUBERCULOUS MYCOBACTERIOSIS

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Abstract [Purpose] This is a retrospective study on relapse/recurrence of surgical cases of pulmonary nontuberculous mycobacteriosis (NTM). Surgical treatment was performed at one hospital and by one surgeon.

[Method] Fifty patients had undergone surgical treatment from August 2004 to July 2011 in hospital. From this group, 37 patients were selected after one year, and of these, 9 patients had a relapse/recurrence (group A) and the others (28 patients without relapse/recurrence, group B). Data was recorded about their age, gender, pre-operative image score, cavernous lesions, residual lesions after operation, drugs of pre-operative chemotherapy, the duration of pre-operative chemotherapy, the duration of any follow-up after operation, type of mycobacteria, the results of bacterial cultivation of surgical specimens, type of mycobacterium and operative procedure.

[Result] Three factors, the result of bacterial cultivation of surgical specimens, duration of chemotherapy before operation and existence of residual lesions, showed a significant difference statistically. No case with major surgical complication and hospital death was recognized.

[Conclusion] The visible foci should be removed as thoroughly as possible. Pre-operative chemotherapy should not be continued unnecessarily, and surgical treatment should be chosen at an early stage. The results of bacterial cultures of surgical specimens could be very useful for predicting the possibility of relapse/recurrence after operation. Surgical treatments of our patients were carried out safely. However, as the patients have a risk of relapse/recurrence, they require careful monitoring and post-operative chemotherapy over a long period.

Key words: Nontuberculous mycobacteriosis (NTM), *Mycobacterium avium* complex (MAC), Surgical treatment, Surgical indication, Relapse, Recurrence

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Case Report

OUTBREAK OF EXTENSIVELY DRUG-RESISTANT PULMONARY TUBERCULOSIS IN A HEMODIALYSIS FACILITY

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Abstract We experienced an outbreak of extensively drug-resistant pulmonary tuberculosis (XDR-TB) in a hemodialysis facility. The primary case involved a 51-year-old male hemodialysis patient, with a history of treatment for *Mycobacterium tuberculosis* infection seven years previously. There was no drug resistance, and the patient completely recovered after undergoing treatment with isoniazid (INH), rifampicin (RFP) and ethambutol (EB). He was admitted to another hospital due to a recurrence of pulmonary tuberculosis in June 2006. At first, he was treated with HRS [INH, RFP and streptomycin (SM)]; however, the drug regimen was changed to INH, EB, levofloxacin (LVFX) and kanamycin (KM) in August following the results of a drug susceptibility test. Although the patient was receiving outpatient tuberculous therapy, he was readmitted in June 2007 due to relapse and conversion of a sputum culture to positive status. Additionally, the XDR-TB organism was identified. Following these events, five staff members of the hemodialysis facility and a member of the patient's family were diagnosed with XDR-TB infection.

The staffs who were infected with XDR-TB had worked in the same dialysis room, drug resistance was found in all cases and drug resistant gene mutations were found in three cases; therefore, we considered this to be an outbreak. As XDR-TB infection was suspected in all cases, no patients took drugs to treat latent tuberculosis infection (LTBI). Regarding the causes of the outbreak, the first is the delay of four months in making a diagnosis of re-exacerbation of tuberculosis. Second, in Case 2, the patient developed laryngeal and tracheobronchial tuberculosis after first being diagnosed with asthma, and the tuberculosis diagnosis was delayed. Third, the sputum smear of Case 2 was strongly positive.

There is only one previously reported outbreak of XDR-TB in Japan; therefore, we consider this outbreak to be educational.

Key words: Multidrug-resistant tuberculosis (MDR-TB), Extensively drug-resistant tuberculosis (XDR-TB), Outbreak, QuantiFERON

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PRIMARY NASOPHARYNGEAL TUBERCULOSIS

Ayaka TAKAGI, Fumihiro NAGAYASU, Yoshimi SUGAMA, and Satoshi SHIRAISHI

Abstract A 59-year-old female was complaining of sore throat, right otorrhea, and hearing impairment. There were no abnormal findings suggestive of pulmonary tuberculosis on her chest XP and CT. Nasopharyngoscopic examination detected a lesion coated with white mass on her nasopharynx, and a biopsy-specimen from this lesion revealed histopathological findings compatible with tuberculosis and the presence of acid-fast bacilli. PCR was positive for *Mycobacterium tuberculosis* complex. Therefore, we diagnosed the case as primary nasopharyngeal tuberculosis and treated her by 4-drug combination regimen with daily isoniazid, rifampicin, ethambutol and pyrazinamide. Later, low degree of resistance was noticed, isoniazid was replaced by levofloxacin. After the anti-tuberculosis chemotherapy, her symptoms almost completely diminished and the mass in her nasopharynx disappeared.

As far as we can search, 23 Japanese cases of primary nasopharyngeal tuberculosis, including this case, have been reported in the literatures. We summarized the clinical features of these cases in Table.

Nasopharyngeal tuberculosis is a rather rare disease. But, recently, due to the advances in diagnostic technology, the number of the case-reports has been increasing. Difficulties in detecting tubercle bacilli in nasopharyngeal lesion sometimes delayed definite diagnosis and treatment. If a patient complains the symptoms compatible with this disease, such as sore throat, pharyngeal pain and otorrhea, which are refractory to the general antibiotic therapy, we should be aware of the existence of this disease and repeat bacteriological and/or molecular examinations to prove tubercle bacilli to be able to start timely anti-tuberculosis chemotherapy.

Key words: Primary nasopharyngeal tuberculosis, Tuberculous otitis media, Anti-tuberculosis agents, Drug resistant tuberculosis

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— Treatment Outcomes —

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Abstract Evaluation of treatment outcomes among tuberculosis (TB) patients using cohort analysis is an essential part of TB control programs. In Japan, treatment outcomes are automatically classified into 15 outcome groups according to a preset computerized algorithm.

The treatment outcomes of new sputum-smear positive pulmonary TB (PTB) cases in 2009 (n＝8,772) were classified as follows: (a) Treatment success (a combination of "Cure" and "Treatment completed"), 51.7%; (b) Dead, 19.3%; (c) Treatment failure, 0.9%; (d) Default, 3.7%; (e) Transfer out, 3.3%; (f) Still on treatment after 12 months, 10.8%; and (g) Unclassified, 10.3%.

Among new sputum-smear positive PTB patients registered in 2007, 2008, and 2009, the proportion removed from the tuberculosis register because of death by the end of the following year was 25.7%, 26.4%, and 26.9%, respectively.

Among the new sputum-smear positive PTB patients registered in 2009 who died within 1 year of treatment commencement (n＝2,102), 37.7% died within 1 month, 52.0% within 2 months, and 62.3% within 3 months.

Key words: Tuberculosis, Sputum-smear positive, Cohort, Treatment outcomes, Success rate, Death

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