──────── **Original Article** ────────

CHRONOLOGICAL DECREASE OF TUBERCULOSIS INCIDENCE RATES BY ORGAN CLASSIFICATION BASED ON A BIRTH COHORT STUDY IN JAPAN, 1975−2005

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Abstract   [Background and objective] The incidence and annual risk of infection of tuberculosis (TB) have decreased rapidly in Japan because of the development of anti-TB medicines and nutritional and hygienic improvements after World War II. The incidence of tuberculosis is currently high among elderly people, reflecting the fact that the prevalence rate of TB infection had been extremely high during their youth. This would suggest that most current cases of TB in the elderly are reactivation of infections acquired in their youth. TB reactivation in various organs have both common and unique aspects. We evaluated the frequency of endogenous reactivation of TB in various organs by examining the TB incidence rate over a 30-year period (1975−2005) in Japan. [Methods] The incidence rate of TB in each organ was obtained for each 10-year birth cohort, using reports of newly registered TB patients in Japan in 1975, 1985, 1995, and 2005. Specifically, the incidence rates of pulmonary TB, lymph node TB, bone-joint TB, kidney TB, and meninges TB were analyzed. [Results] Chronological changes in TB incidence rates in each organ were characterized by a rapidly declining phase followed by a stationary phase in every organ TB except pulmonary TB. Incidence rates among the already infected population in the stationary phase were 3.0 (lymph node TB), 1.2 (bone-joint TB), 0.5 (kidney TB), and 0.3 (meninges TB) per 100,000 cases, respectively. [Conclusions] Once infected with TB, the incidence rate of TB in these organs does not decrease below the above-mentioned values.
Key words : Tuberculosis, Incidence rate, Reactivation, Extra-pulmonary TB

—————— **Original Article** ——————

DOCTOR'S DELAY IN ENDOBRONCHIAL TUBERCULOSIS

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Abstract [Objective] The aim of this study was to investigate the current status of doctor's delay in diagnosing endobronchial tuberculosis (EBTB) and to elucidate the risk factors contributing to the delay. [Methods] Retrospective clinicopathological analysis. [Patients] Sixty-two patients with EBTB were admitted at our hospital between 1999 and 2010. Their backgrounds, symptoms, diagnoses at initial consultation, delay in diagnosis, and clinical examination results were analyzed. [Results] Of the 62 patients, 59 had acid-fast, bacilli-positive sputum smear test results at admission. Among the 40 patients with total diagnostic delay of more than 2 months, only 11 experienced long patient's delay exceeding 2 months. However, 22 patients experienced long doctor's delay of more than 2 months (28% vs. 55%, respectively, $p < 0.05$), suggesting that doctor's delay contributes more to total delay than patient's delay. Fever was less frequent in patients with long doctor's delays than in those without (0% vs. 18%, respectively), at the initial consultation. In addition, radiographs showed that patients with long doctor's delays more frequently presented with shadows in the lower lung field (50% vs. 23%, $p < 0.05$), and most of these patients had noncavitary shadows on admission. All 7 patients diagnosed with bronchial asthma at the initial consultation had long doctor's delays. [Conclusion] These findings demonstrate that long doctor's delays in diagnosing EBTB remain an issue. The clinical features of EBTB with long doctor's delays were confirmed to be quite different from those of pulmonary tuberculosis.

Key words: Endobronchial tuberculosis, Doctor's delay, Radiographic findings, Bronchial asthma

───────Memorial Lecture by the Imamura Award Winner───────

GENETIC RESEARCH ABOUT
MYCOBACTERIUM AVIUM COMPLEX

Kenji OGAWA

Abstract   We conducted four genetic studies on the *Mycobacterium avium* complex (MAC). (1) *M.avium* genotyping : A total of 70 clinical isolates from patients with pulmonary MAC infections were typed by MATR-VNTR, IS*1245*-RFLP, and MIRU-VNTR analyses to compare discriminatory powers of these typing methods. To allow a comparison of discriminatory powers, the Hunter-Gaston discriminatory index (HGDI) was calculated, giving a HGDI of 0.960 for IS*1245*-RFLP, 0.949 for MIRU-VNTR, and 0.990 for MATR-VNTR, demonstrating that MATR-VNTR analysis is the best of the three genotyping methods. (2) Genetic characteristics of *M.avium* : Japanese clinical isolates of *M.avium* were subjected to insertion sequence (IS) analyses. First, an analysis of 81 isolates by heat shock protein 65 identified all isolates as belonging to the subspecies of *M.avium* subsp. *hominissuis*. Another analysis by IS*901* identified about 70％ of the isolates as IS*901*-carriers. IS*901* had been thought to be carried by the subspecies that infect birds : *M.avium* subsp. *avium* and *M.avium* subsp. *silvaticum*. Studies have reported that most human isolates in the U.S. and Europe carry no IS*901*. The prevalence of IS*901*-carriers among Japanese clinical isolates of *M.avium* is thus a significant characteristic. A further analysis of the IS*901* showed that compared with *M.avium* subsp. *avium*, the clinical isolates shared 60 point mutations of nucleotide sequence. This novel insertion sequence was designated "IS*Mav6*". (3) The CAM-resistance gene in MAC : This study assessed the correlation between CAM-susceptibility and mutation of the gene involved in drug resistance (A DNA sequence analysis identified mutations at positions 2058 and 2059 in domain V of 23S-rRNA). Furthermore, a system was developed to rapidly detect the presence/absence of CAM resistance by ARMS-PCR, a procedure used to detect gene mutations. The utility of this new system was also evaluated. A total of 253 clinical isolates were tested for drug susceptibility, with 227 isolates identified as sensitive and 26 as resistant. Sequence analyses showed that all 28 strains randomly selected for testing from the sensitive strains were wild type, whereas 24 of the 26 resistant strains were mutant type. The rest of the 2 strains were subsequently confirmed to be mutant type after they were isolated from contaminations with sensitive strains. These results showed an association between drug susceptibility and drug-resistant gene mutation. In addition, ARMS-PCR provided a sensitivity of 84.6％ (22/26) and a specificity of 100％ (28/28) for the detection of gene mutations. The lower sensitive was probably attributable to the fact that every one of the 4 strains was a combination of wild type and mutant type. These results indicated that compared with drug-susceptibility tests, ARMS-PCR provides earlier results on the presence/absence of drug resistance and has the capability of rapid detection even when the specimen contains a mixture of sensitive and resistant strains. (4) Development of a VNTR analysis for *M.intracellulare* : Bioinformatics analyses were used to develop a VNTR analysis for *M.intracellulare* and to evaluate the utility of the VNTR analysis. First, the Tandem Repeat Finder (TRF) software was used to conduct a search of TR loci on the genomic data of *M.intracellulare* ATCC 13950 published in December 2007, resulting in the identification of 16 TR loci, which were used in VNTR analyses of 74 isolates from pulmonary MAC infections. The HGDI was 0.988, suggesting an excellent discriminatory power. Furthermore, a stability evaluation of the VNTR loci was conducted in isolates from patients with long-term bacilli discharge. The VNTR loci were stable without changes for up to 4 years in 14 such patients. These results indicated that this method is useful in *M.intracellulare* genotyping and in determining whether the cause of recurrence in recurred patients is endogenous from the remnant bacilli or exogenous from another infection of different bacilli, given that the VNTR loci have been confirmed to be stable.

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[Japanese original : Kekkaku, 2011 ; 86 : 61−68]

──────── **Memorial Lecture by the Imamura Award Winner** ────────

DISEASE PROGRESSION OF *MYCOBACTERIUM AVIUM* PULMONARY INFECTION AND THE MYCOBACTERIAL VARIABLE NUMBER TANDEM REPEAT (VNTR) TYPING

Toshiaki KIKUCHI

Abstract : Nontuberculous mycobacteriosis may progress to fatal chronic respiratory infections. Some cases remain stable over a relatively long period of time. With no well established progression predictors yet available, we conducted a retrospective analysis of the association between mycobacterial variable numbers of tandem repeat (VNTR) and clinical progression in 37 patients who were seen at the Department of Respiratory Medicine, Tohoku University Hospital between 2005 and 2006 and from whose respiratory tract specimens *M.avium* was isolated and cultured. The disease type in the 15 patients who began an antimicrobial therapy within 1 year after a bacteriological diagnosis was defined as progressive, and that in the 9 patients who began an antimicrobial therapy 2 years or longer after diagnosis was defined as stable. A cluster analysis of the mycobacterial VNTR genotypes showed concentrations of the progressive-type isolates and the stable-type isolates in different clusters. Furthermore, the study demonstrated that multiple logistic regression analysis can be used to construct a model for estimating, with statistical significance, progression of nontuberculous mycobacteriosis based on the mycobacterial VNTR genotype. These results indicated that whether a nontuberculous mycobacteriosis is progressive can be estimated by the VNTR genotyping of the nontuberculous mycobacterium.

Key words : Nontuberculous mycobacteriosis, Treatment standard, Minisatellite repeat, Computational biology, Cluster analysis