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Effect of drug resistance on negative conversion of sputum culture in patients with pulmonary tuberculosis

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SUMMARY

Objective: Negative conversion of sputum culture is a useful marker for predicting treatment outcome and relapse of pulmonary tuberculosis (TB). The effect of drug resistance on negative conversion of sputum culture with treatment was evaluated in this study.
Methods: A total of 535 patients with culture-proven pulmonary TB were classified into three groups: drug-susceptible (DS), other drug-resistant (ODR), and multidrug-resistant/extensively drug-resistant (MDR/XDR). Rates of conversion of sputum culture at 4, 8, and 12 weeks were compared.
Results: At 4 weeks of treatment, the conversion rate in the DS group (52.7%) was higher than that in the ODR group (30.8%, $p = 0.003$), but was not different compared with the MDR group (45.7%, $p = 0.427$). At 8 weeks, the conversion rate in the DS group (76.3%) was higher than that in the ODR (63.5%, $p = 0.042$) and MDR groups (60.0%, $p = 0.031$). At 12 weeks, the conversion rate in the DS group (85.9%) tended to be higher than that in the MDR group (74.3%, $p = 0.062$), but was not different from that in the ODR group (84.6%, $p = 0.796$).
Conclusion: The pattern of resistance to anti-TB drugs affects culture conversion rates in the early phase of treatment and also prolongs the time to culture conversion.

1. Introduction

Despite the reduction in incidence and prevalence of tuberculosis (TB) through efforts worldwide, TB remains a global health problem. In 2013, 9.0 million new cases of TB, 1.5 million deaths among HIV-negative people with TB, and 360 000 deaths among HIV-positive people with TB were reported.[1]

To monitor the outcomes of treatment, sputum should be obtained for microscopic examination and culture at monthly intervals until two consecutive culture specimens are negative and also at the completion of treatment.[2] The sputum culture-negative conversion rate after 2 months of anti-TB treatment is a reliable surrogate marker for relapse after the completion of treatment.[3] In addition, a delay in conversion is associated with the risk of acquiring drug resistance.[4] Among patients with multidrug-resistant (MDR) TB, those who have early conversion to negative results show better treatment outcomes than patients with delayed culture conversion.[5]

Resistance to anti-TB drugs, including mono-resistance, multidrug resistance, and extensive drug resistance, causes worse treatment outcomes.[6,7] However, the difference in rate of conversion at certain time points or the time to conversion in TB patients with varying resistance to anti-TB drugs remains unclear. Previous studies on this issue have had limitations, such as a small number of patients or the absence of a certain drug resistance group.[8–10] Therefore, the aim of this study was to determine the effect of the drug resistance pattern on the monthly conversion rate of sputum culture, as well as the time to culture conversion.

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2. Methods

2.1. Patients

This retrospective cohort study included all consecutive patients who were diagnosed with culture-proven pulmonary TB and treated with the standard World Health Organization (WHO) regimen between January 1, 2009 and December 31, 2012. The patients were from Seoul National University Hospital, a tertiary referral hospital in South Korea. Patients for whom drug susceptibility test results were not available and patients who were missing the results of mycobacterial culture of sputum at 8 weeks after the initiation of anti-TB treatment were excluded. The study was approved by the ethics committee of the Seoul National University Hospital Institutional Review Board.

2.2. Data collection

Patient demographic data, as well as past medical history and co-morbidities, were reviewed and recorded. The results of acid-fast staining and mycobacterial culture of sputum before and during treatment, drug susceptibility test results, and details of the treatment regimen were also collected.

2.3. Laboratory processing of specimens

For all specimens, an acid-fast bacillus (AFB) smear was performed using auramine–rhodamine staining and confirmed by Ziehl–Neelsen staining. Culture was performed in Ogawa medium for 8 weeks in 5–10% CO_2 incubators and in BACTEC MGIT for 6 weeks. When cultured, the isolation of *Mycobacterium tuberculosis* was confirmed using the Seeplex MTB/NTM ACE detection method (Seegene, Seoul, South Korea).

The mycobacteriology laboratory of the study institution is a biosafety level 3 facility (BSL-3; with inward directional airflow and double-door entry). Two biosafety cabinets are used (both class II, type A2), and the processing and handling of mycobacterial colonies is limited to one cabinet. For pipetting, filter tips are always applied and two or more tube caps are not allowed to be opened at one time. After work is completed for the day, ultraviolet irradiation is applied to the inside of the biological safety cabinet and the entire mycobacteriology laboratory until the next morning.

Although mycobacterial cultures were performed at Seoul National University Hospital, drug susceptibility testing was performed at the Korean Institute of Tuberculosis (Osong, South Korea), which is the supranational reference laboratory for TB. Resistance to each drug that was tested was defined as $\geq$1% bacterial growth in Löwenstein–Jensen medium. The absolute concentration method was used, with the following critical concentrations of anti-TB drugs: isoniazid 0.2 μg/ml, streptomycin 10.0 μg/ml, ethambutol 2.0 μg/ml, rifampicin 40.0 μg/ml, para-aminosalicylic acid 1.0 μg/ml, prothionamide 40.0 μg/ml, cycloserine 30.0 μg/ml, kanamycin 40.0 μg/ml, capreomycin 40.0 μg/ml, ofloxacin 2.0 μg/ml, levofloxacin 2.0 μg/ml, and moxifloxacin 2.0 μg/ml. Pyrazinamide susceptibility was determined using the pyrazinamidase test.[11]

2.4. Definitions and comparisons

Patients were classified into three groups according to the pattern of drug resistance: susceptible to all first-line anti-TB drugs (drug-susceptible (DS) group), presence of mono- or poly-resistance to first-line drugs but not meeting the definition for MDR-TB (other drug-resistant (ODR) group), and MDR-TB, including extensively drug-resistant (XDR) TB (MDR/XDR group). Definitions of MDR-TB and XDR-TB were those given in the WHO guidelines.[2] Negative culture conversion was defined as two consecutive negative sputum cultures in liquid medium. The date of conversion was defined as the date of first negative culture satisfying culture conversion. Negative sputum culture followed by contaminated culture was also regarded as culture conversion if a subsequent culture gave a negative result. If patients could not expectorate sputum after one occurrence of negative sputum culture, this was also defined as culture conversion.

The rate of conversion at 4, 8, and 12 weeks of anti-TB treatment and the time to conversion of sputum were compared among the three groups. A 1-week period was allowed for each time point if results of mycobacterial culture of sputum were unavailable at the predefined time points of 4, 8, and 12 weeks. The time to conversion was defined as the number of days from initiation of anti-TB treatment to the date of conversion. In the MDR/XDR group, the time to conversion was counted from the date of initiation of second-line anti-TB drugs.

Treatment outcomes were classified based on guidelines published by the WHO for drug-susceptible TB, as well as drug-resistant TB.[12] Being 'transferred' or 'still on treatment' was determined at the time of writing the manuscript.

2.5. Statistical analysis

Baseline demographic and clinical characteristics are summarized using descriptive statistics, such as the median and interquartile range (IQR). For comparisons between groups, *p*-values obtained using Pearson's Chi-square test, one-way analysis of variance, or the Wilcoxon–Mann–Whitney rank sum test, as appropriate, are reported. The Kaplan–Meier method and log-rank test were used to compare the time to conversion. A *p*-value of <0.05 was considered statistically significant. All analyses were conducted using Stata Statistics version 13 (StataCorp, College Station, TX, USA).

3. Results

3.1. Baseline characteristics of the patients

A total of 535 patients were included in the study and analyzed. Of these, 448 (83.7%) patients were allocated to the DS group, 52 (9.7%) to the ODR group, and 35 (6.5%; 28 MDR patients and seven XDR patients) to the MDR/XDR group. Patients in the MDR/XDR group were younger (median 41 years) than those in the other groups ($p = 0.002$). Patients in the MDR/XDR group were more likely to have a history of previous TB treatment (55.9%, $p < 0.001$) and a positive AFB sputum smear at the time of diagnosis (48.6%, $p = 0.008$). Baseline characteristics of the patients are shown in Table 1.

Patients in the DS group were more likely to achieve cure, while those in the MDR/XDR group were more likely to be transferred or were still on treatment. In the DS group, 404 (90.2%) patients were classified as cured or completed treatment. In the MDR/XDR group, 27 (77.1%) patients were classified as having successful treatment (Table 2). The number of patients still on treatment was two in the DS group, one in the ODR group, and two in the MDR/XDR group.

3.2. Comparison of culture conversion rates at 4, 8, and 12 weeks of treatment

At the end of the first 4 weeks of treatment, the rate of conversion in the DS group (52.7%) was higher than that in the ODR group (30.8%, $p = 0.003$), but was not different from that in the MDR/XDR group (45.7%, $p = 0.427$). At 8 weeks, the proportion of patients with negative conversion in the DS group increased to