Early Discontinuation of Ethambutol in Pulmonary Tuberculosis Treatment Based on Results of the GenoType MTBDRplus Assay: a Prospective, Multicenter, Noninferiority Randomized Trial in South Korea

Kyung-Wook Jo,^a Mihye Kim,^b Ye-Jee Kim,^c Hyun-Kyung Lee,^d Hyun Kuk Kim,^e Doosoo Jeon,^f Jiwon Lyu,^g Hye Kyeong Park,^h Jeongha Mok,ⁱ Ju Sang Kim,^j Eun Young Heo,^k Sang Bong Choi,^l Jae-Joon Yim,^m Tae Sun Shim^a

^aDivision of Pulmonology and Critical Care Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul, South Korea

^bPulmonary, Allergy and Critical Care Medicine, Gangneung Asan Hospital, Gangneung, South Korea

^cDepartment of Clinical Epidemiology and Biostatistics, Asan Medical Center, Seoul, South Korea

^dDivision of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Inje University Busan Paik Hospital, Busan, South Korea

^eDepartment of Pulmonary and Critical Care Medicine, Haeundae Paik Hospital, Inje University College of Medicine, Busan, South Korea

^fDepartment of Internal Medicine, Pusan National University Yangsan Hospital, Yangsan, South Korea

^gDivision of Respiratory Medicine, Department of Internal Medicine, Soonchunhyang University Cheonan Hospital, Cheonan, South Korea

^hDivision of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Inje University Ilsan Paik Hospital, Inje University College of Medicine, Ilsan, South Korea

ⁱDepartment of Internal Medicine, Pusan National University Hospital, Busan, South Korea

^jDivision of Pulmonary and Critical Care Medicine, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Incheon, South Korea

^kDivision of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Seoul Metropolitan Government-Seoul National University Boramae Medical Center, Seoul National University College of Medicine, Seoul, South Korea

^lDepartment of Internal Medicine, Sanggye Paik Hospital, Inje University College of Medicine, Seoul, South Korea

^mDivision of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Seoul National University College of Medicine, Seoul, South Korea

ABSTRACT No studies have investigated whether the discontinuation of ethambutol (EMB) based on susceptibility to isoniazid and rifampin, as determined by the GenoType MTBDRplus assay, would be appropriate. We aimed to determine the feasibility of discontinuing EMB before the end of the intensive phase of treatment based on the results of the MTBDRplus assay in patients with pulmonary tuberculosis (PTB). This prospective, multicenter noninferiority randomized trial was conducted at 12 referral centers in South Korea in drug-susceptible PTB patients who initiated the standard four-drug regimen for PTB. Based on the results of the assay, EMB was discontinued in the MTBDRplus group after confirmation that the *M. tuberculosis* isolate was susceptible to isoniazid and rifampin. The time point for EMB discontinuation in the guideline group was determined using the results of the phenotypic drug susceptibility test based on the South Korean national tuberculosis guidelines. The primary outcome was treatment success. Secondary outcomes included the 1-year rates of recurrence and adverse events. Of 600 randomized patients, the treatment outcome analysis was performed for 493 patients (MTBDRplus group, 244 patients; guideline group, 249 patients). Treatment success rates were 93.9% (229/244) in the MTBDRplus group and 93.6% (233/249) in the guideline group and did not differ between groups (relative risk, 1.00; 95% confidence interval, 0.95 to 1.06). The 1-year recurrence rates in the two groups (0.9% and 0.5%, respectively) and adverse drug reactions did not differ between the groups. In conclusion, the early discontinuation of EMB based on the results of the MTBDRplus assay did not affect the treatment outcomes in patients with PTB. (This clinical trial has been registered with the Clinical Research Information Service of the Republic of Korea [<https://cris.nih.go.kr/cris/>] under registration no. KCT0000610.)

Citation Jo K-W, Kim M, Kim Y-J, Lee H-K, Kim HK, Jeon D, Lyu J, Park HK, Mok J, Kim JS, Heo EY, Choi SB, Yim J-J, Shim TS. 2019. Early discontinuation of ethambutol in pulmonary tuberculosis treatment based on results of the GenoType MTBDRplus assay: a prospective, multicenter, noninferiority randomized trial in South Korea. *Antimicrob Agents Chemother* 63:e00980-19. <https://doi.org/10.1128/AAC.00980-19>.

Copyright © 2019 American Society for Microbiology. All Rights Reserved.

Address correspondence to Tae Sun Shim, shimts@amc.seoul.kr.

K.-W.J. and M.K. contributed equally to this article.

Received 13 May 2019

Returned for modification 23 June 2019

Accepted 31 August 2019

Accepted manuscript posted online 16 September 2019

Published 21 November 2019

KEYWORDS MTBDRplus assay, ethambutol, tuberculosis, treatment outcome, recurrence

The standard regimen for tuberculosis (TB) is a 2-month treatment with isoniazid (INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA), followed by a 4-month therapy with INH and RIF (1). The role of EMB in the four-drug regimen is to prevent the development of acquired resistance beyond INH in settings where the level of INH resistance among new TB cases is high and INH susceptibility testing is not done (or results are not available) (2). Accordingly, once a *Mycobacterium tuberculosis* isolate is confirmed to be susceptible to INH and RIF by drug susceptibility testing (DST), EMB has no further role in the treatment regimen and therefore can be discontinued. Because the prevalence of INH resistance is approximately 10% in South Korea and the time to detection of drug resistance is delayed by the use of solid media in DST (3), the South Korean national TB guidelines recommend the inclusion of EMB even during the continuation phase of the treatment (4). Accordingly, attending physicians in South Korea discontinue EMB after phenotypic DST results confirm drug susceptibility, usually during the continuation phase. Otherwise, in real-world clinical practice, they often continue to use EMB until the end of the treatment, regardless of the DST results.

The median interval from the initiation of treatment to the confirmation of INH and RIF susceptibility by solid medium-based DST to the requesting clinician is reported to be 80 to 90 days in South Korea (5, 6). That is, a relatively long period of time passes after treatment initiation before a physician can determine whether EMB can be discontinued. However, recently developed molecular tests have enabled the rapid identification of susceptibility to core anti-TB drugs. The GenoType MTBDRplus assay (Hain Lifescience, Nehren, Germany), one such test, is reported to be sensitive and specific for the detection of INH and RIF resistance (7). Using this assay, the median interval between the start of anti-TB therapy and the confirmation of results was reported to be 19.8 days in a study conducted in South Korea (6), which is a significant improvement over the interval when traditional solid medium-based DST is used. Thus, rapid determination of INH and RIF susceptibility using the MTBDRplus assay could allow for the early discontinuation of EMB in the course of anti-TB treatment. Because no studies have investigated this matter, we aimed to determine the feasibility of discontinuing EMB during the intensive phase of pulmonary TB treatment based on MTBDRplus assay results, before phenotypic DST results are available. We expect that the duration of EMB treatment could be dramatically shortened if the outcomes of a treatment strategy based on the MTBDRplus assay are acceptable.

RESULTS

Study subjects. Patient enrollment began in November 2012 and ended in January 2016; the 1-year follow-up after the end of the treatment was completed in February 2018. After screening, 600 patients were enrolled and underwent randomization into the intention-to-treat (ITT) population in the study (Fig. 1). Of these patients, 358 (59.7%) received no anti-TB treatment at the time of enrollment, whereas the remaining 242 (40.3%) were under treatment for <14 days. Of the 600 patients, the MTBDRplus assay was directly performed on 323 (53.8%) specimens with a positive acid-fast bacillus (AFB) smear (164 in the MTBDRplus group [in which treatment was initiated with the standard four-drug treatment and the decision to discontinue EMB was based on the MTBDRplus assay results] and 159 in the guideline group [in which treatment was initiated with the standard four-drug treatment and the decision to discontinue EMB was based on phenotypic DST results]). For the remaining 277 (46.2%) patients with a negative AFB smear, the assay was carried out with a positive culture isolate (136 in the MTBDRplus group and 141 in the guideline group).

Treatment outcome. Of the 600 patients in the ITT group, treatment outcome analysis was performed on 493 (82.2%) patients in the modified ITT (mITT) group with culture-confirmed, drug-susceptible TB who initiated the standard anti-TB medication

FIG 1 Study flowchart. DST, drug susceptibility testing. *, the reasons for exclusion are as follows: final acid-fast bacillus culture growth of a nontuberculous mycobacterium ($n = 4$), the standard antituberculosis regimen change was not initiated due to cancer chemotherapy ($n = 3$), HIV infection ($n = 1$), no growth of *Mycobacterium tuberculosis* ($n = 1$), and the patient was determined to be ineligible ($n = 1$).

(MTBDRplus group, 244 patients; guideline group, 249 patients; Fig. 1). The demographic and clinical characteristics of the patients were similar between the two groups (Table 1).

As shown in Fig. 2, treatment success was achieved in 93.9% (229/244) of the patients (in which the patients were cured [$n = 155$] or treatment was completed [$n = 74$]) in the MTBDRplus group, whereas treatment success was achieved in 93.6% (233/249) of the patients (in which the patients were cured [$n = 162$] and treatment was completed [$n = 71$]) in the guideline group ($P = 0.898$), for a relative risk of 1.00

TABLE 1 Baseline characteristics of 493 patients in the modified intention-to-treat group

Characteristic	Value for the following group:			P value
	Total ($n = 493$)	MTBDRplus ($n = 244$)	Guideline ($n = 249$)	
Mean $\pm$ SD age (yr)	55.8 $\pm$ 17.0	55.4 $\pm$ 17.1	56.2 $\pm$ 17.0	0.574
No. (%) of male patients	291 (59.0)	142 (58.2)	149 (59.8)	0.711
Mean $\pm$ SD body mass index (kg/m ²)	22.0 $\pm$ 3.2	21.9 $\pm$ 3.1	22.1 $\pm$ 3.2	0.340
No. (%) of patients who were a current or past smoker	212 (43.0)	105 (43.0)	107 (43.0)	0.989
No. (%) of patients with a history of tuberculosis	69 (14.0)	35 (14.3)	34 (13.7)	0.825
No. (%) of patients with the following comorbidity:				
Diabetes mellitus	71 (14.4)	36 (14.8)	35 (14.1)	0.825
Malignancy	50 (10.1)	24 (9.8)	26 (10.4)	0.824
Chronic lung disease ^a	18 (3.7)	9 (3.7)	9 (3.6)	0.965
Chronic liver disease	10 (2.0)	6 (2.5)	4 (1.6)	0.541
On immunosuppressants	2 (0.4)	2 (0.8)	0	0.244
No. (%) of patients with a positive AFB ^b smear at treatment initiation	277 (56.2)	141 (57.8)	136 (54.6)	0.478
No. (%) of patients with the presence of a cavity	121 (24.5)	58 (23.8)	63 (25.3)	0.693
No. (%) of patients with the following radiological severity:				0.903
Minimal	381 (77.3)	188 (77.0)	193 (77.5)	
Moderate to far advanced	112 (22.7)	56 (23.0)	56 (22.5)	

^aChronic lung disease included chronic obstructive pulmonary disease and bronchial asthma.

^bAFB, acid-fast bacillus.

FIG 2 Treatment outcomes for 493 patients in the modified intention-to-treat population according to the study group.

(95% confidence interval [CI], 0.95 to 1.06). Since the lower limit of the 95% CI for the relative risk between the two groups did not exceed the noninferiority margin of 0.95, we concluded that the MTBDRplus group was noninferior to the guideline group at the 2.5% significance level. Unfavorable treatment outcomes were not different between the two groups: 6.1% (15/244) of patients in the MTBDRplus group (in which the patients died [$n = 8$], were not evaluated [$n = 3$], or were lost to follow-up [$n = 2$] or in whom treatment failed [$n = 2$]) and 6.4% (16/249) of patients in the guideline group (in which the patients died [$n = 6$], were not evaluated [$n = 6$], or were lost to follow-up [$n = 4$]). The results of treatment outcome analysis in the per-protocol population were similar to those in the mITT population (see Table S1 in the supplemental material).

Treatment duration, time to culture conversion, and culture conversion rate.

While the total treatment duration and the duration of the intensive phase were similar between the two groups, as expected, there was a significant difference in the duration of EMB treatment, with a median duration of 14.0 days (interquartile range, 9.0 to 16.0 days) and 75.0 days (interquartile range, 62.0 to 98.0 days) in the MTBDRplus group and the guideline group, respectively ($P < 0.001$; Table 2). For 39 (15.7%) of the 249 patients in the guideline group, EMB was prescribed for the entire treatment period. Both groups showed a similar time to culture negativity and a similar culture conversion rate after treatment initiation (Fig. 3; Table 3).

One-year recurrence. Of the 462 successfully treated patients, 440 (MTBDRplus group, 218 patients; guideline group, 222 patients) were followed up for 1 year after the end of the treatment. The reasons for the exclusion of the remaining 22 patients after the end of successful treatment included loss to follow-up ($n = 13$) and death ($n = 9$). Of the 440 patients, 3 (0.7%) experienced a recurrence within 1 year after successful treatment. The recurrence rate did not differ between the two groups, with recurrences

TABLE 2 Treatment duration for 493 patients according to study group

Group	Treatment duration ^a (days)		
	Total	Ethambutol	Intensive phase
Total ($n = 493$)	200.8 $\pm$ 59.4	27.0 (14.0–75.0)	65.2 $\pm$ 19.9
MTBDRplus ($n = 244$)	205.3 $\pm$ 73.4	14.0 (9.0–16.0)	65.7 $\pm$ 22.2
Guideline ($n = 249$)	196.6 $\pm$ 41.9	75.0 (62.0–98.0)	64.8 $\pm$ 17.3
<i>P</i> value	0.301	< 0.001	0.635

^aData are reported as the mean $\pm$ standard deviation or the median (interquartile range).

FIG 3 Kaplan-Meier estimates of the distribution of the time to sputum culture conversion over 6 months in the modified intention-to-treat population according to the study group.

occurring in 0.9% (2/218) of the patients in the MTBDRplus group and 0.5% (1/222) of the patients in the guideline group ($P = 0.552$). Restriction fragment length polymorphism (RFLP) analysis performed for paired initial and recurrent culturable *M. tuberculosis* isolates from one patient showed identical RFLP patterns for the two episodes, indicating endogenous reactivation (Fig. S1).

Adverse drug events and ophthalmologic examination. Analysis of the incidence of adverse drug events and the rate of occurrence of an ophthalmologic examination was performed for all 600 patients who underwent randomization. We found no significant between-group differences in the incidence of adverse drug reactions, which occurred in 30 patients (10.0%) in the MTBDRplus group and 31 patients (10.3%) in the guideline group (Table S2). Of the various adverse drug reactions, hepatotoxicity was the most common. In addition, the number of patients who received an ophthalmologic examination was significantly lower in the MTBDRplus group than in the guideline group (Table S2).

Performance of the MTBDRplus assay. Of the 600 enrolled patients, 538 were compared with respect to the rates of resistance to INH and RIF between assessments via phenotypic DST and the MTBDRplus assay. Of the 538 samples, 518 (96.3%) showed an identical pattern of drug resistance between the two methods and 15 were identified to be multidrug-resistant TB (MDR-TB) using phenotypic DST (Table S3). The sensitivity of the MTBDRplus assay for detecting INH resistance, RIF resistance, and MDR-TB was 86.0%, 94.7%, and 100.0%, respectively; the specificity was 97.8%, 98.6%, and 99.2%, respectively (Table S4).

DISCUSSION

Recently developed molecular tests, such as the MTBDRplus assay used in this study, can rapidly preclude drug-resistant TB, raising the possibility that the use of EMB could be adjusted early in the course of TB treatment. To our knowledge, this is the first study to investigate the feasibility of discontinuing EMB based on the results of the

TABLE 3 Time to culture negativity and culture conversion rate after treatment initiation for 493 patients according to study group

Group	Median (IQR ^a) time to sputum culture conversion (days)	Culture conversion rate (%) ^b at:				
		2 mo	3 mo	4 mo	5 mo	6 mo
Total ($n = 493$)	58.0 (36–84)	52.8	77.4	89	92.8	95.9
MTBDRplus ($n = 244$)	56.0 (36–91)	55	74.2	88.1	91.4	94.7
Guideline ($n = 249$)	60.0 (36–77)	50.9	80.2	91.5	94	97
<i>P</i> value	0.933	0.468	0.197	0.62	0.368	0.3

^aIQR, interquartile range.

^bThe analysis of the sputum culture conversion rate after treatment initiation was performed for 318 of 493 patients (61.8% [151/244] in the MTBDRplus group and 67.0% [167/249] in the guideline group) in the modified intention-to-treat group. This was because the data for monthly sputum culture results after treatment initiation over 6 months after treatment initiation were available for these 318 patients.

MTBDRplus assay during the intensive phase, before phenotypic DST results are available. Among patients with culture-confirmed drug-susceptible pulmonary TB whose treatment was initiated with the standard regimen, we found no difference in treatment outcomes or the incidence of recurrences between those assessed for the early discontinuation of EMB using the results of the MTBDRplus assay and those assessed using the results of phenotypic DST.

Theoretically, EMB can be discontinued once susceptibility to INH, RIF, and PZA has been confirmed (8). However, the guidelines differ regarding the recommended timing for discontinuing EMB after DST results are available. According to the WHO guidelines, which were also adopted for the national TB guidelines in South Korea, the initial phase should consist of 2 months of INH, RIF, PZA, and EMB, and the continuation phase should consist of 4 months of INH and RIF for drug-susceptible TB (2). These guidelines therefore recommend that EMB be included during the intensive phase even if the *M. tuberculosis* isolate is confirmed to be susceptible to INH and RIF (4). In contrast, American Thoracic Society, Centers for Disease Control and Prevention, and Infectious Diseases Society of America clinical practice guidelines for the treatment of drug-susceptible TB (9) and Canadian TB standards (10) state that EMB can be discontinued as soon as the DST results demonstrate that the isolate is susceptible to INH and RIF. Results from the current study reiterate the North American position that during the intensive phase, EMB can be discontinued immediately after susceptibility of *M. tuberculosis* to INH and RIF is confirmed, albeit more rapidly by the MTBDRplus assay than by DST.

However, it is uncertain whether the strategy of early EMB discontinuation based on the MTBDRplus assay results is applicable to all patients with drug-susceptible TB. Previous studies have shown that the total number of *M. tuberculosis* bacilli varies according to the severity of TB (11). In other words, patients with more extensive pulmonary TB have more *M. tuberculosis* bacilli in their lungs. Because *M. tuberculosis* resistance is caused by spontaneous mutation, the treatment of extensive TB with two or even three drugs might lead to the development of acquired drug resistance as a result of spontaneous mutations (10). In contrast, two effective drugs are adequate for many cases of nonextensive pulmonary TB. In the present study, we could not determine whether EMB can be discontinued based on the MTBDRplus assay results for a patient with extensive disease, such as a patient with bilateral lung involvement with multiple cavities and slow sputum bacteriologic conversion. Among 493 patients in the mITT group, 51 (23 in the MTBDRplus group and 28 in the guideline group) had cavities with moderate to far advanced involvement and delayed sputum culture conversion (more than 3 months after treatment initiation). Although the treatment success rate (100% in the MTBDRplus group and 96.4% in the guideline group) among such patients was comparable to that among the remaining patients in the mITT group, the small sample size of patients with these characteristics prevents us from drawing a reliable conclusion. Further research is needed to elucidate the optimal duration of EMB for treating extensive drug-susceptible TB with delayed culture conversion.

A previous study in South Korea showed that the specificity of the MTBDRplus assay in detecting INH resistance was very high (98.3%), although the sensitivity was not optimal (93.3%) (6). In the present study, the sensitivity and specificity of the MTBDRplus assay for detecting INH resistance were 86.0% and 97.8%, respectively. Therefore, the physician should check the results of phenotypic DST, even if the *M. tuberculosis* isolate is reported to be INH susceptible by the MTBDRplus assay. In addition, considering that the performance of the MTBDRplus assay in detecting INH resistance could be dependent on regional differences in the frequency of specific mutations causing the resistance phenotype (12–15), the results of the present study should be interpreted with caution in countries in which the mutation frequencies of the genes associated with INH resistance differ from those in South Korea.

Among the 300 patients in the ITT group whose EMB was discontinued immediately based on MTBDRplus assay results, 3 had phenotypic DST results indicating resistance to one of the antituberculosis drugs (Fig. 1). The treatment regimens of these three

patients were changed after the attending physician noted the DST results. Two of these three patients were followed up, and both were successfully treated with a subsequent regimen. The detailed characteristics, regimens, and treatment outcomes for these three patients are shown in Table S5 in the supplemental material. This result suggests that the results of the MTBDRplus assay must be confirmed by DST after adjusting the medication based on the MTBDRplus assay results. These findings also suggest that the treatment outcome may be favorable if the anti-TB medication is properly adjusted according to the DST results, even several weeks after treatment initiation.

EMB-induced optic neuropathy is a well-known complication of EMB administration, the severity of which is dose dependent (16). A systematic review showed that the incidence of all visual impairments and permanent visual impairments associated with EMB treatment was 19.2 and 2.3 persons per 1,000 patients, respectively, when the patients were treated with the standard dose for 2 to 9 months (17). Yang et al. reported that 3 of 415 (0.7%) South Korean TB patients developed toxic optic neuropathy over a 6-year period (18). According to the Canadian guidelines (10), patients taking EMB for longer than just the initial phase should be referred to an ophthalmologist for periodic assessment. In addition, monthly nursing assessment of visual acuity and red-green color discrimination is recommended. Since there is no specific recommendation for periodic ophthalmologic examinations in the South Korean national TB guidelines (4), such examinations were performed at the discretion of the attending physician in the present study. Our findings show that the treatment strategy of early EMB discontinuation based on the results of the MTBDRplus assay may not only reduce the possibility of EMB-induced optic neuropathy but also circumvent the need to receive an ophthalmologic examination (Table S3).

This study has several limitations of note. First, the power was lower than expected for the sample size, as we did not obtain our target population. The sample size obtained during the study included less than the target number determined by our power calculations for primary outcome analysis. With the sample size obtained, we achieved a 53.5% power for primary outcome analysis between the two groups. Second, because this was not a double-blind study, significant biases could be present. For example, it was possible that patients in the MTBDRplus group were treated for a longer duration, thereby reducing the incidence of recurrences and enhancing the treatment success rate; however, there was no significant difference in the treatment duration between the two groups. Third, adherence to the treatment was not confirmed by directly observed treatment (DOT) because the South Korean national TB guidelines have not yet adopted DOT. Despite monitoring the treatment course of patients at all institutions by private-public mix nurses (19), we cannot eliminate the possibility of the noncompliance of some patients.

In conclusion, we showed that the early discontinuation of EMB based on the MTBDRplus assay results did not alter the treatment success or recurrence rate in patients with culture-confirmed drug-susceptible pulmonary TB whose treatment was initiated with the standard regimen from the treatment success or recurrence rate in those for whom the decision to discontinue EMB was based on phenotypic DST results. These findings suggest that if the MTBDRplus assay is available for the rapid detection of resistance and shows the susceptibility of the *M. tuberculosis* strain, EMB can be excluded from the standard four-drug regimen during the intensive phase. Considering that EMB is often prescribed in South Korea for the entire treatment period, even in patients with drug-susceptible pulmonary TB, we believe that the results of this study may help reduce unnecessary treatment with EMB.

MATERIALS AND METHODS

Study design and participants. This prospective, multicenter, noninferiority randomized trial was conducted in 12 referral centers in South Korea between November 2012 and February 2018. The treatment outcomes of pulmonary TB patients were compared between those for whom EMB discontinuation was based on the MTBDRplus assay results and those for whom discontinuation was based on

phenotypic DST results. The trial protocol was approved by the institutional review boards at all sites. All patients provided written informed consent before enrollment.

Adult patients (≥ 18 years of age) with (i) culture-confirmed pulmonary TB or (ii) a positive sputum AFB smear with imaging findings consistent with pulmonary TB were screened. We included patients who had not yet received treatment for TB or who had been under treatment for less than 2 weeks. Exclusion criteria were as follows: (i) a history of TB resistant to INH and/or RIF; (ii) an inability to receive the standard four-drug treatment because of an underlying condition, such as liver disease; (iii) any condition that may prove fatal during the course of anti-TB treatment, such as advanced-stage cancer; (iv) combined extrapulmonary TB requiring prolonged treatment (e.g., TB meningitis); and (v) imaging findings compatible with typical findings of lung disease due to a nontuberculous mycobacterium (20), despite a positive AFB smear, in cases for which the AFB culture result was not yet available.

Randomization. Randomization was centralized using an online, computer-generated randomization system before examining the MTBDRplus assay results. Patients were stratified into two groups according to the presence of cavitation (21). In addition, they were further stratified according to AFB smear positivity. Eligible patients were randomized in a 1:1 ratio to one of the following therapies: (i) treatment initiation with the standard four-drug treatment comprising INH, RIF, EMB, and PZA and with the decision to discontinue EMB based on the MTBDRplus assay results (MTBDRplus group) or (ii) treatment initiation with the standard four-drug treatment and with the decision to discontinue EMB based on phenotypic DST results (guideline group).

Study procedure. In the MTBDRplus group, the study protocol indicated that the attending physician should discontinue EMB as soon as the *M. tuberculosis* isolate was reported to be both INH and RIF susceptible based on the MTBDRplus assay results. According to the study protocol, this decision should be made within 4 weeks after treatment initiation. In the guideline group, the decision to discontinue EMB was based on the phenotypic DST result, regardless of the MTBDRplus assay result. South Korean national TB guidelines state that the discontinuation of EMB can be considered during the continuation phase for drug-susceptible TB. Therefore, in the guideline group, EMB could be (i) discontinued immediately after the phenotypic DST result was identified or (ii) prescribed during the whole treatment period, according to the attending physician's decision. All other routine anti-TB treatments were similar for both groups. The total treatment duration was determined by each attending physician. After initial screening and baseline visits, the interval of follow-up visits and examinations, including laboratory tests and sputum AFB smear/culture, was determined by the attending physician at each institution, but these were usually at approximately 1-month intervals. In addition, each institution determined whether to perform ophthalmologic examinations, such as visual acuity or visual field assessments, during the treatment. Patients with treatment success received follow-up examinations at 6 and 12 months after the end of the treatment. For patients identified as having no evidence of a 1-year recurrence after treatment success, the study was considered ended.

Outcomes. The primary outcome was the proportion of patients who achieved treatment success at the end of the treatment. The proportion of patients who achieved treatment success was defined as the sum of the proportion who were cured and the proportion who completed treatment. An unfavorable treatment outcome included (i) treatment failure, (ii) death, (iii) loss to follow-up, and (iv) a lack of evaluation. All treatment outcome categories were in accordance with the revised 2013 WHO definitions (22). Secondary outcomes included (i) the 1-year recurrence rate; (ii) the rate of adverse events, defined as any adverse drug reaction requiring transient discontinuation or the need for a permanent change of at least one anti-TB drug in the regimen; (iii) the number of patients who underwent ophthalmologic examination; and (iv) the total and intensive-phase treatment duration, EMB treatment duration, time to culture conversion, and culture conversion rate in each of the two groups. After patient enrollment, we performed an interim analysis of the primary and secondary outcomes every 6 months to identify whether the treatment outcomes of the two groups were comparable.

MTBDRplus assay and bacteriological study. The MTBDRplus assay was performed at each center according to the manufacturer's instructions. This assay was requested for all specimens with a positive AFB smear. In the case of a negative sputum smear, the MTBDRplus assay was requested for those positive for an isolate by culture.

AFB smears were examined by Ziehl-Neelsen staining, and the degree was classified using the quantitation scale suggested by the American Thoracic Society (23). AFB culture was carried out using both solid and liquid media. Phenotypic DST was performed using the absolute concentration method with Löwenstein-Jensen medium at the Korean Institute of Tuberculosis, South Korea's TB reference laboratory. The wells contained the following critical concentrations of anti-TB drugs: 0.2 $\mu\text{g/ml}$ for INH and 40 $\mu\text{g/ml}$ for RIF. Growth exceeding that in the control wells by $>1\%$ was considered to indicate drug resistance.

Statistical analysis. The study was designed as a noninferiority trial. We assumed that the rate of treatment success for the standard four-drug regimen in the guideline group was 95.0%, based on previous studies (1). We set the noninferior margin ratio, with the group ratio between the two groups being 0.95. To estimate the sample size for noninferiority testing, a ratio of 0.95 was used with a power of 0.80 and a significance level (alpha value) of 0.025. As such, if the treatment success rate in the MTBDRplus group was 90.3%, under the null hypothesis (H_0) of noninferiority to the standard treatment (H_0 , MTBDRplus group/guideline group ≤ 0.95 ; alternative hypothesis [H_1], MTBDRplus group/guideline group = 1.00 > 0.95), then a sample size of 357 patients per group would be needed (PASS; NCSS statistical software, 2011, Kaysville, UT, USA). The final sample size was 400 patients per group, allowing for a 10% dropout rate. The relative risk and asymptotic (Wald) confidence limits were estimated, assuming a confidence coefficient of 95%.

Because the key assumption of our study was targeted to culture-confirmed drug-susceptible pulmonary tuberculosis (PTB) under a standard treatment and the results of the MTBDRplus assay were available only after randomization, the treatment outcome analysis (including the treatment success rate and the 1-year recurrence rate) was performed for the mITT population rather than the entire randomized ITT population. Patients were categorized into the mITT population, after excluding from the ITT population (i) those with *M. tuberculosis* isolates resistant to anti-TB drugs by either the MTBDRplus assay or DST, (ii) those whose MTBDRplus assay result was not interpretable, (iii) those whose final AFB culture results revealed no growth of *M. tuberculosis* or the growth of a nontuberculous mycobacterium, and (iv) those who did not initiate the standard anti-TB treatment for various reasons (Fig. 1).

The baseline characteristics and treatment data were compared between the two groups using the chi-square test or Fisher's exact test for categorical variables and Student's *t* test or the Mann-Whitney *U* test for continuous variables, as appropriate. All tests of significance were two-sided, and a *P* value of <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS software (version 20.0; IBM Corp., Armonk, NY, USA) and SAS software (version 9.4; SAS Institute, Inc., Cary, NC, USA).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00980-19>.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

ACKNOWLEDGMENTS

This research was supported by funds from the Korea Centers for Disease Control and Prevention and the Korean Institute of Tuberculosis (grant 2012-KIT-004).

The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had the final responsibility for the decision to submit for publication.

We have no conflicts of interest to declare.

Kyung-Wook Jo, Mihye Kim, and Tae Sun Shim designed the study. Kyung-Wook Jo, Mihye Kim, Ye-Jee Kim, Hyun-Kyung Lee, Hyun Kuk Kim, Doosoo Jeon, Jiwon Lyu, Hye Kyeong Park, Jeongha Mok, Ju Sang Kim, Eun Young Heo, Sang Bong Choi, Jae-Joon Yim, and Tae Sun Shim analyzed the data. Kyung-Wook Jo, Mihye Kim, Ye-Jee Kim, and Tae Sun Shim wrote the manuscript. All authors contributed to the study design, interpretation of the data, and the writing of the manuscript. All authors had full access to the data and reviewed/approved the manuscript.

REFERENCES

- Shin HJ, Kwon YS. 2015. Treatment of drug susceptible pulmonary tuberculosis. *Tuberc Respir Dis* (Seoul) 78:161–167. <https://doi.org/10.4046/trd.2015.78.3.161>.
- World Health Organization. 2009. Treatment of tuberculosis guidelines. <http://www.who.int/tb/publications/2010/9789241547833/en/>. Accessed 13 May 2019.
- Bai GH, Park YK, Choi YW, Bai JI, Kim HJ, Chang CL, Lee JK, Kim SJ. 2007. Trend of anti-tuberculosis drug resistance in Korea, 1994–2004. *Int J Tuber Lung Dis* 11:571–576.
- Tuberculosis Treatment Guidelines Revised Committee. 2017. Korean tuberculosis treatment guidelines. <http://www.lungkorea.org/bbs/index.html?code=guide&category=&gubun=&page=1&number=7563&mode=view&keyfield=&key=>. Accessed 13 May 2019.
- Joh JS, Lee CH, Lee JE, Park YK, Bai GH, Kim EC, Han SK, Shim YS, Yim JJ. 2007. The interval between initiation of anti-tuberculosis treatment in patients with culture-positive pulmonary tuberculosis and receipt of drug-susceptibility test results. *J Korean Med Sci* 22:26–29. <https://doi.org/10.3346/jkms.2007.22.1.26>.
- Lyu J, Kim MN, Song JW, Choi CM, Oh YM, Lee SD, Kim WS, Kim DS, Shim TS. 2013. GenoType(R) MTBDRplus assay detection of drug-resistant tuberculosis in routine practice in Korea. *Int J Tuber Lung Dis* 17: 120–124. <https://doi.org/10.5588/ijtld.12.0197>.
- Ling DI, Zwerling AA, Pai M. 2008. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. *Eur Respir J* 32:1165–1174. <https://doi.org/10.1183/09031936.00061808>.
- Horsburgh CR, Jr, Barry CE, III, Lange C. 2015. Treatment of tuberculosis. *N Engl J Med* 373:2149–2160. <https://doi.org/10.1056/NEJMra1413919>.
- Nahid P, Dorman SE, Alipanah N, Barry PM, Brozek JL, Cattamanchi A, Chaisson LH, Chaisson RE, Daley CL, Grzemska M, Higashi JM, Ho CS, Hopewell PC, Keshavjee SA, Lienhardt C, Menzies R, Merrifield C, Narita M, O'Brien R, Peloquin CA, Raftery A, Saukkonen J, Schaaf HS, Sotgiu G, Starke JR, Migliori GB, Vernon A. 2016. Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America clinical practice guidelines: treatment of drug-susceptible tuberculosis. *Clin Infect Dis* 63:e147–e195. <https://doi.org/10.1093/cid/ciw376>.
- Government of Canada. 2014. Canadian tuberculosis standards. <https://www.canada.ca/en/public-health/services/infectious-diseases/canadian-tuberculosis-standards-7th-edition.html>. Accessed 13 May 2019.
- Canetti G, Grosset J. 1961. Percentage of isoniazid-resistant and streptomycin-resistant variants in wild strains of *Mycobacterium tuberculosis* on Loewenstein-Jensen medium. *Ann Inst Pasteur (Paris)* 101: 28–46. (In French.)
- Lacoma A, Garcia-Sierra N, Prat C, Ruiz-Manzano J, Haba L, Roses S, Maldonado J, Dominguez J. 2008. GenoType MTBDRplus assay for molecular detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* strains and clinical samples. *J Clin Microbiol* 46:3660–3667. <https://doi.org/10.1128/JCM.00618-08>.
- Anek-Vorapong R, Sinthuwattanawibool C, Podewils LJ, McCarthy K, Ngamlert K, Promsarin B, Varma JK. 2010. Validation of the GenoType MTBDRplus assay for detection of MDR-TB in a public health laboratory in Thailand. *BMC Infect Dis* 10:123. <https://doi.org/10.1186/1471-2334-10-123>.
- Barnard M, Albert H, Coetzee G, O'Brien R, Bosman ME. 2008. Rapid

- molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. *Am J Respir Crit Care Med* 177:787–792. <https://doi.org/10.1164/rccm.200709-1436OC>.
15. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. 2015. Genetic mutations associated with isoniazid resistance in *Mycobacterium tuberculosis*: a systematic review. *PLoS One* 10:e0119628. <https://doi.org/10.1371/journal.pone.0119628>.
 16. Song W, Si S. 2017. The rare ethambutol-induced optic neuropathy: a case-report and literature review. *Medicine (Baltimore)* 96:e5889. <https://doi.org/10.1097/MD.0000000000005889>.
 17. Ezer N, Benedetti A, Darvish-Zargar M, Menzies D. 2013. Incidence of ethambutol-related visual impairment during treatment of active tuberculosis. *Int J Tuberc Lung Dis* 17:447–455. <https://doi.org/10.5588/ijtld.11.0766>.
 18. Yang HK, Park MJ, Lee JH, Lee CT, Park JS, Hwang JM. 2016. Incidence of toxic optic neuropathy with low-dose ethambutol. *Int J Tuberc Lung Dis* 20:261–264. <https://doi.org/10.5588/ijtld.15.0275>.
 19. Park JS. 2011. Increasing the treatment success rate of tuberculosis in a private hospital through public-private mix (PPM) project. *Tuberc Respir Dis* 70:143–149. <https://doi.org/10.4046/trd.2011.70.2.143>.
 20. Yuan MK, Chang CY, Tsai PH, Lee YM, Huang JW, Chang SC. 2014. Comparative chest computed tomography findings of non-tuberculous mycobacterial lung diseases and pulmonary tuberculosis in patients with acid fast bacilli smear-positive sputum. *BMC Pulm Med* 14:65. <https://doi.org/10.1186/1471-2466-14-65>.
 21. Benator D, Bhattacharya M, Bozeman L, Burman W, Catanzaro A, Chaisson R, Gordin F, Horsburgh CR, Horton J, Khan A, Lahart C, Metchock B, Pachucki C, Stanton L, Vernon A, Villarino ME, Wang YC, Weiner M, Weis S. 2002. Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomised clinical trial. *Lancet* 360:528–534. [https://doi.org/10.1016/S0140-6736\(02\)09742-8](https://doi.org/10.1016/S0140-6736(02)09742-8).
 22. World Health Organization. 2013. Definitions and reporting framework for tuberculosis. Revision. http://apps.who.int/iris/bitstream/10665/79199/1/9789241505345_eng.pdf. Accessed 13 May 2019.
 23. Anonymous. 2000. Diagnostic standards and classification of tuberculosis in adults and children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. *Am J Respir Crit Care Med* 161(4 Pt 1):1376–1395. <https://doi.org/10.1164/ajrccm.161.4.16141>.