Factors Associated With Misdiagnosis of Smear-Negative Tuberculosis: An Experience in Taiwan

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BACKGROUND: A negative sputum smear from a patient with history, physical examination, and chest x-ray findings suggestive of tuberculosis (TB) presents a diagnostic dilemma. We investigated the possible factors associated with a misdiagnosis and inappropriate treatment of TB among such patients. METHODS: We reviewed the records of 193 patients whose diagnoses with TB included conflicting test results and were reported to the Taiwan Centers for Disease Control in 2004. When other conditions were found to underlie the initial abnormal chest x-ray finding, the diagnosis was revised. RESULTS: *Mycobacterium tuberculosis* was isolated from sputum samples in 72 of 193 patients (37%), nontuberculous mycobacteria from 4 (2%), and no bacteriologic evidence of *M. tuberculosis* from 117 (61%). The initial diagnosis of TB was revised for 26 (13.5%) patients. Patients with positive *M. tuberculosis* culture had a lower incidence of revised diagnoses (4.2%, $P < .001$) than those negative for mycobacterial culture (17.1%) and those with nontuberculous mycobacteria (75%). Chest cavitations in this study were not a significant predictor of revised diagnosis (odds ratio 0.30, $P = .08$). CONCLUSIONS: An incorrect diagnosis of TB despite a negative sputum smear result is more likely to be made for patients positive for nontuberculous mycobacteria culture and less likely for patients with positive *M. tuberculosis* culture. *Key words: acid-fast smear; Asia; cavity formation; nontuberculous; tuberculosis; misdiagnosis.* [Respir Care 2012;57(5):753–757. © 2012 Daedalus Enterprises]

Introduction

Tuberculosis (TB) is a major public health problem, affecting almost one third of the world's population.[1] In 2005, 8.8 million new cases of TB and 1.6 million deaths were reported, making it the second most common cause of infectious disease death.[2] TB is also a substantial problem in Taiwan. In 2004, the Taiwan Centers for Disease Control (CDC) reported that 16,784 Taiwanese individuals had diagnoses of TB, an incidence of 74 in 100,000.[3]

The diagnosis of TB is challenging, as reflected by the fact that more than half of all cases globally remain undetected.[2] Sputum culture for acid-fast bacilli is a relatively reliable and robust means of detecting TB, and rates of positive identification as high as 80% have been reported.[4] However, the culture process is time consuming and not always feasible in lower income countries. Sputum smear microscopy is, therefore, the most widely used TB

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diagnostic technique. Although this test is low in cost, specific, and rapid, specimens from as few as 20–50% of patients with TB produced positive results with this technique.[5] Hence, false-negative results for smears from patients with pulmonary TB are an important problem.[6]

The rate of smear-positive results for new cases of pulmonary TB in Taiwan in 2005 was a worryingly low 38%. We speculate that poor specimen collection, early testing, and incorrect diagnosis of pulmonary TB by other means may have contributed to this low rate of smear-positive results.

The World Health Organization criteria for TB diagnosis include positive culture for *Mycobacterium tuberculosis*; acid-fast bacillus test, which can be used in case culturing is unavailable; clinical characteristics; radiographic examination; and evaluation by physicians. The TB symptoms should also improve after treatment. However, misdiagnosis can happen under at least these 5 conditions:

- Low-grade positivity of sputum smear due to lower bacillary burden was more frequent in females and extremes of age.[7]
- Propensity for false negatives of sputum smears may arise from inadequate smears and improper reading of the slides,[8] and additional training can improve the accuracy.[9]
- The possibility of laboratory cross-contamination also could cause the misdiagnosis of TB.[10]
- Simultaneous infection with human immunodeficiency virus, common in TB patients, also tends to increase the likelihood of a negative smear result.[11,12]
- Sometimes the radiographic abnormality cannot differentiate between many non-tuberculous respiratory diseases and pulmonary TB, due to the similar symptoms and signs; these diseases were often misdiagnosed as pulmonary TB with negative smear.[13]

For whatever reason, a false-negative smear result is a definite concern because, while patients with smear-negative TB have lower bacillary burdens and minimal pulmonary lesions, these patients are capable of actively transmitting the disease.[14] Thus, a patient with a history, physical examination, and chest x-ray findings suggestive of TB, but a negative smear, presents a treatment dilemma. While it is not prudent to delay treatment until culture results are available, early treatment will inevitably result in some patients receiving unwarranted treatment.

Elucidation of any characteristics commonly found among Taiwanese patients with negative smear results and an initial incorrect diagnosis of TB would have clinical utility. The presence of such characteristics could alert physicians to a higher possibility of a misdiagnosis or over-diagnosis. Hence, in this study we retrospectively examined the records of patients with negative sputum smear results who were initially treated for TB but who were subsequently found not to have TB (on the basis of acid-fast bacilli culture or lack of response to treatment) to identify any characteristics that could be useful in differentiating patients with negative sputum smears who do not have TB from those who do.

QUICK LOOK

Current knowledge

A negative sputum smear in patients with a history and physical findings suggestive of tuberculosis creates a diagnostic dilemma. Misdiagnosis may be associated with inappropriate treatment and complications.

What this paper contributes to our knowledge

The presence of positive non-tuberculosis mycobacteria is associated with the misdiagnosis of tuberculosis. Cavitary lesions on chest radiograph were not a predictor of revised diagnosis.

Methods

Patients and Study Design

We retrospectively reviewed all records of 394 patients in the Chest Hospital, Department of Health, Taiwan, and selected those patients who had negative acid-fast smear results but chest x-ray or other findings (ie, persistent cough, weight loss, fever, or improved symptoms after antibiotic therapy) suggestive of TB and for whom new diagnoses of TB were reported to the Taiwan CDC in 2004. We excluded individuals from other countries and patients with extra-pulmonary TB or human immunodeficiency virus infection. Finally, 201 patients were excluded from this study. For each of 193 analyzed participants, information pertaining to demographics, mycobacterial culture, mycobacteria identification (ie, definitive diagnosis), and chest x-ray findings had been recorded and standardized. For analysis of age as a potential confounding factor, participants were originally stratified by age into 4 groups: < 40 years, 41 to < 60 years, 60 to < 80 years, and ≥ 80 years of age, but, due to the size of the groups, the groups were consolidated and analyses were performed using 2 groups: patients < 60 years old, and those ≥ 60 years of age.

This study was reviewed and approved by the Chest Hospital Committee on Medical Science and Education.

TB Diagnostic Policies

In 2004, acid-fast bacilli smear and culture were performed simultaneously on each patient's samples in our hospital.

Radiographic Analysis

X-ray images were read and interpreted by 2 independent physicians in pulmonary medicine. If no concordant decision was made, a third physician was consulted to resolve the difference.

Laboratory Procedures

Sputum samples were decontaminated with the sodium hydroxide-*N*-acetyl-L-cysteine method, as previously detailed.[15] Cytocentrifugation, smear preparation, and microscopic examination of Ziehl-Neelsen-stained sputum smears were performed in accordance with standard methods.

Parallel Löwenstein-Jensen and 7H10 cell cultures were carried out with the remainder of the specimen. After inoculation, samples were incubated at 37°C and examined daily for a week and then weekly through day 64 of culture. Identification of *Mycobacteria* was performed by traditional biochemical methods at a laboratory certified by the Taiwan CDC.

Definition of Incorrect Diagnosis

The initial diagnosis of TB was considered incorrect and was revised when other conditions were determined to have caused the abnormal chest x-ray finding and the culture results were negative for *M. tuberculosis*. Other conditions included malignancy, pneumonia, infection with nontuberculous mycobacteria, or fibrosis. A diagnosis of pneumonia was made in cases when the patient died soon after diagnosis or the patient's condition improved and *M. tuberculosis* was not isolated. A diagnosis of malignancy was indicated by pathologic or cytologic findings when *M. tuberculosis* was not isolated. A diagnosis of nontuberculous mycobacteria lung disease or nontuberculous mycobacteria contamination was made in cases where the culture finding was positive for nontuberculous mycobacteria but *M. tuberculosis* was not isolated. The diagnosis of TB was also considered incorrect and revised if the patient's sputum culture result was positive for *M. tuberculosis* but the patient's doctor revised the diagnosis clinically and thought that the *M. tuberculosis* isolation was due to contamination. The diagnosis of fibrosis was made if fibrosis was apparent, no improvement was noted after treatment had been administered for more than 2 months, and no *M. tuberculosis* was isolated.

Statistical Analysis

Demographics and characteristics are presented as frequency (percentage). Chi-square or the Fisher exact test was used to compare variables between patients in whom a diagnosis of TB was confirmed or shown to be incorrect, and revised as defined above. Univariate and multivariate logistic regression analyses were performed to identify factors that were more common among patients whose diagnoses were incorrect (revised). Both unadjusted and adjusted odds ratios (OR) and 95% CI were determined. Statistical analyses were performed with software (SPSS 15.0, SPSS, Chicago, Illinois), and a significance level of $\alpha = .05$ was used.

Results

This retrospective analysis examined the records of 193 patients who met the study inclusion criteria. Demographic and clinical characteristics of the 193 participants and the groups of patients with and those without revised diagnoses are summarized in Table 1. The diagnosis of TB was shown to be incorrect and was revised in 26 (13.5%) patients: 2 of 52 patients $<$ 40 years old (3.8%), 9 of 50 patients who were between 40 and 60 years old (18.0%), and 15 of 91 patients $\geq$ 60 years old (16.5%). Among these 26 patients, the diagnosis of TB was revised to lung cancer for 12, pulmonary fibrosis for 8, pneumonia for 4, and nontuberculous mycobacteria lung disease for 2 (data not shown).

The percentage of patients for whom the diagnosis of TB was revised was 42.3% (11/26) in age $<$ 60 years, and 57.7% (15/26) in age $\geq$ 60 years ($P = .25$). A lower proportion of patients with a positive *M. tuberculosis* culture had a revised diagnosis than those with a correct diagnoses (11.5% vs 41.3%, $P < .001$). Most patients with revised diagnoses were negative for mycobacterial culture (76.9% vs 58.1%, $P < .001$). Three of the 72 patients (4.16%) with positive *M. tuberculosis* culture, 3 of the 4 patients (75%) with positive culture results for nontuberculous mycobacteria, and 20 of the 117 patients (17.1%) with negative culture results had their diagnoses revised.

Multivariate logistic regression analysis assessed the effect of age, mycobacterium culture results, and chest x-ray findings on the probability of a revised diagnosis (Table 2). A positive *M. tuberculosis* culture finding generally confirmed the diagnosis of TB and was therefore a significant negative predictor of the need for diagnostic revision (OR 0.22, $P = .02$). In contrast, a positive nontuberculous mycobacteria culture result was a significant positive predictor of an incorrect diagnosis and the need to check for revision (OR 14.61, $P = .03$). However, chest cavitations after adjustment for age in multiple logistical regression analysis did not become a significant predictor of revised diagnosis (OR 0.30, $P = .08$).

Discussion

In this study we sought to elucidate any characteristics that were associated with revision of TB diagnoses in patients with a negative sputum smear result, but with other

Table 1. Demographic and Clinical Characteristics of Patients With Negative Acid-Fast Bacilli Smears Included in the Study

Characteristic	Overall (n = 193)	Diagnosis Revised (no. = 26)	Diagnosis Not Revised (no. = 167)	P
Age, y*				
< 60	102 (52.8)	11 (42.3)	91 (54.5)	.25
≥ 60	91 (47.2)	15 (57.7)	76 (45.5)	
Sex†				
Men	124 (64.2)	17 (65.4)	107 (64.1)	.90
Women	69 (35.8)	9 (34.6)	60 (35.9)	
Mycobacterium Culture*				
Mycobacterium tuberculosis	72 (37.3)	3 (11.5)	69 (41.3)	< .001
Nontuberculous mycobacteria	4 (2.1)	3 (11.5)	1 (0.6)	
Negative	117 (60.6)	20 (76.9)	97 (58.1)	
Chest X-ray Findings*				
Cavitations	53 (27.5)	3 (11.5)	50 (29.9)	.059
No cavitations	140 (72.5)	23 (88.5)	117 (70.1)	

Data are expressed as number (percentage).
* Data were compared by Fisher exact test.
† Data were compared by chi-square test.

Table 2. Multiple Logistic Regression Analysis of Factors Associated With Revised Diagnosis of Tuberculosis (n = 193)

Characteristic	Odds Ratio (95% CI)	P
Age, y		
≥ 60	1.74 (0.71–4.26)	.23
< 60	1	
Mycobacterium Culture		
Mycobacterium tuberculosis	0.22 (0.06–0.77)	.02
Nontuberculous mycobacteria	14.61 (1.33–160.41)	.03
Negative	1	
Chest X-ray Findings		
Cavitations	0.30 (0.08–1.16)	.08
No cavitations	1	

clinical findings suggestive of TB. We found that an incorrect diagnosis was more likely in patients who were positive for nontuberculous mycobacteria culture and less likely for patients with positive *M. tuberculosis* culture. Chest cavitation was not a significant predictor of revised diagnosis, although the number of observed cases was small. Our findings confirmed that misdiagnosis according to the TB diagnosis criteria by the World Health Organization occasionally happened, and our results can be used as a comparison for accuracy of future fast diagnosis.

Among the 193 patients with negative sputum smear results included in this study, *M. tuberculosis* was isolated from 72 (37%). Positive culture results from patients with negative sputum smears have ranged from around 10%[15] to 88% in a study in which the natural course of smear-negative TB was evaluated in a chemotherapy trial in Hong Kong.[16] Some of this variability is undoubtedly the result of differences in the quality of clinical laboratories as well as factors related to the patients. The laboratory in which the analysis was performed for our study is accredited by the Taiwan CDC, which should minimize the occurrence of misdiagnoses resulting from laboratory errors. Nevertheless, we found that the diagnoses had been revised in 3 patients with positive *M. tuberculosis* culture results, because these patients did not respond to treatment. The positive findings in these particular cases may have been the result of laboratory errors, such as cross-contamination of culture specimens.[17]

Our finding that 24 of the 26 patients who had their diagnosis revised were older than 40 years of age is consistent with the observation that the prevalence of nontuberculous mycobacteria lung disease and malignancy are higher in older patients.[18] Both of these conditions have been reported to mimic features of TB in chest x-ray images and, thus, increase the possibility of misdiagnosis of TB.[19,20] One of the 2 patients younger than 40 years of age in our study who had their diagnosis revised was found to have a tumor, while the other was found to have pneumonia, despite the fact that *M. tuberculosis* was isolated (presumably because of sample contamination). Nontuberculous mycobacteria was not isolated from any patient younger than 40 years of age. These results emphasize the fact that physicians should be especially cautious in making a diagnosis of TB in older patients who have negative results from acid-fast bacillus smear.

Chest cavitations upon x-ray examination were significantly more likely to receive correct initial diagnoses of *M. tuberculosis*. Wilcke et al previously reported that 89% of patients

with radiographic evidence of chest cavities had positive acid-fast smears, while 53% of patients who did not have chest cavities had positive smear results.[21] The authors recommended that further diagnostic procedures should be performed to rule out an alternative diagnosis in patients with chest cavitations but negative smear findings.

In our hospital, the 2011 policy states that all patients whose conditions are suggestive of TB be tested by polymerase chain reaction, TB culture, smear from sputum, bronchoscopic brushing and lavage, and chest computed tomography. This policy takes a more active approach to TB diagnosis than the policy in 2004, when the patients in this study were examined. Therefore, our data from this study of patients in 2004 can serve as a baseline for comparison in future studies of the efficacy of the newer TB diagnostic policies.

This study is limited by the relatively small number of patients and the study's retrospective nature, which allowed access only to previously collected data. Unfortunately, records of some patients did not include assessments of symptoms (cough, night sweats, lymphadenopathy, and laboratory results), and the incomplete dataset reduced the statistical power of analyses. Only 2 patients in the group age < 40 years had the outcome of interest (revised diagnosis); nevertheless, the difference in outcomes reached statistical significance. The study was conducted in a single hospital, which is the only first-line diagnosis and treatment center for TB in southern Taiwan. Therefore, caution should be used in applying our results generally to other institutions. Furthermore, we included only patients with smear-negative results in the study because the diagnosis for these patients is more difficult. However, this inclusion criterion necessarily limits the relevance of our results to patients with negative sputum smears. In some instances, only a single smear study was performed before treatment, which contrasts with the general recommendation that 2 or 3 smears be tested before a smear-negative diagnosis is made. The single smear analysis would, therefore, tend to increase the rate of false-negative smear results.

Conclusions

Our study showed that patients who had a negative sputum smear result and were positive for nontuberculous mycobacteria culture were more likely to have an incorrect TB diagnosis than those with positive *M. tuberculosis* cultures. Our findings suggest that positive nontuberculous mycobacteria cultures are predictors of revised TB diagnosis. Chest cavitations were not a significant predictor of revised diagnosis. These results emphasize the fact that physicians should be especially cautious in making a diagnosis of TB in patients who have negative results from acid-fast bacillus smear and positive nontuberculous mycobacteria cultures.

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