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Acceptability of Sputum Specimens for Diagnosing Pulmonary Tuberculosis

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The evaluation of the quality of a sputum specimen prior to bacterial culture has been an accepted practice. However, optimal sputum criteria for pulmonary tuberculosis (TB) are not well established. We investigated indicators for sputum acceptability in tuberculosis cultures and acid-fast bacilli (AFB) smear. A post-hoc analysis of a randomized trial with 228 sputum specimens from 77 patients was conducted. In the trial, pulmonary TB suspects were requested for collecting three sputum specimens. We performed both TB study (AFB smear and *M. tuberculosis* culture) and Gram staining in each specimen. By using generalized estimating equations, the association between sputum characteristics and positive TB testings were analyzed. Although acceptable specimens for bacterial pneumonia showed higher TB-culture positive rates than unacceptable specimens (adjusted odds ratio [aOR] = 1.66; 95% confidence interval [CI] = 1.11-2.49), a specimen with ≥ 25 white blood cells/low-power field was the better predictor for positive *M. tuberculosis* cultures (aOR = 2.30; 95% CI = 1.48-3.58) and acid-fast bacilli smears (aOR = 1.85; 95% CI = 1.05-3.25). Sputum leukocytosis could be an indicator of sputum acceptability for diagnosing pulmonary tuberculosis.

Keywords: Acceptable Sputum; Tuberculosis, Pulmonary; Sputum WBC

INTRODUCTION

Sputum specimens containing ≥ 10 leukocytes with mucus, but < 25 squamous epithelial cells per low-power field (LPF, × 100), are unlikely to be contaminated by oropharyngeal flora and considered suitable for bacterial culture in patients with suspected bacterial pneumonia (1, 2).

According to the pulmonary tuberculosis (TB) guidelines, patients require instructions regarding the proper method of sputum collection (3). Patients need to be informed that a desired sputum specimen consists of material brought up from the lungs after a productive cough and not nasopharyngeal discharge or saliva. However, there is no clear consensus regarding the indicators for appropriate sputum specimens in diagnosing pulmonary TB. Furthermore, it has not been fully evaluated whether the sputum criteria for bacterial pneumonia is helpful for determining acceptable sputum specimens for diagnosing pulmonary TB. However, saliva-containing sputum specimens contribute to the diagnosis of TB (4, 5). Good indicators for the 'acceptable' specimen that can predict microbiological diagnosis of TB has not been fully evaluated.

We previously conducted a randomized trial to evaluate whether educating patients with a simple brochure was beneficial for obtaining an appropriate sputum sample for effectively diagnosing pulmonary TB (6). Herein, we investigated indicators for sputum acceptability in *M. tuberculosis* cultures.

MATERIALS AND METHODS

The randomized trial was conducted from January 2009 to July 2010 at a single center. Patients with suspected pulmonary TB were randomly allocated to 2 groups on the basis of whether they received the simple brochure with instructions on sputum collection: brochure group vs. non-brochure group. Patients were requested to collect 3 sputum specimens on consecutive days (6). Each sputum specimen was divided into 2 for the TB study (AFB smear and *M. tuberculosis* culture) and Gram staining. According to the Gram staining results, the specimens were graded using the Murray and Washington's system. Sputum specimens of grade 4 or 5 were considered acceptable for diagnosing bacterial pneumonia (1, 7-9).

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Statistical analysis

We performed repeated measures logistic regression analyses using generalized-estimating equation (GEE) models (Stata command: xtgee) adjusted by age, sex, and brochure group (vs. the non-brochure group) to elucidate whether the sputum criteria are independently associated with positive *M. tuberculosis* cultures and positive AFB smears. The identification number of each participant was defined as the variable of repetitions. Statistical significance was determined at $P < 0.05$. All analyses were performed using Stata 13.1 software (StataCorp, Texas, USA).

Ethics statement

The original study protocol (6) was approved by the institutional review board of Seoul Metropolitan Government Seoul National University Boramae Medical Center (number: 20090602/06-2009-73/84) and conducted in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Table 1. Characteristics of included patients and sputum specimen results for diagnosing pulmonary tuberculosis

Variables	Values
Patients, No.	77
Sputum specimens, No.	228
mean (± SD)	2.96 (± 0.49)
Age, median (range)	55 (18-88)
Male, No. (%)	54 (70.1)
History of tuberculosis, No. (%)	27 (35.1)
History of smoking, No. (%)	
Non-smoker	48 (62.3)
Current smoker	18 (23.4)
Ex-smoker	11 (14.3)
Cavitary lesion in X-ray/CT	20(26.0)
Positive TB culture	
Sputum, No. (%)	78 (34.2)
Patients, No. (%)	31 (40.3)
Positive AFB smear	
Sputum, No. (%)	58 (25.4)
Patients, No. (%)	25 (32.5)

TB, tuberculosis; AFB, acid-fact bacilli; SD, standard deviation.

RESULTS

In total, 228 sputum specimens from 77 patients were analyzed. Approximately 34.2% of sputum specimens and 40.3% of patients were diagnosed with culture positive pulmonary TB; 25.4% of sputum specimens and 32.5% of patients showed positive AFB smears (Table 1).

Sputum specimens with grade 3-5 were independent predictors of positive *M. tuberculosis* cultures in GEE models (grade 3: adjusted adds ratio [aOR], 2.84; 95% confidence interval [CI], 1.43-5.64; grade 4: aOR, 2.98; 95% CI, 1.47-6.06; grade 5: aOR, 3.38; 95% CI, 1.67-6.87). Therefore, we defined the sputum criteria for TB as specimens with grade 3-5 and a white blood cell (WBC) count of ≥ 25 cells/LPF.

The sputum criteria for bacterial pneumonia (grade 4 or 5) were significantly associated with positive *M. tuberculosis* cultures (aOR, 1.66; 95% CI, 1.11-2.49), but not positive AFB smears (aOR, 1.43; 95% CI, 0.87-2.37). The sputum criteria for TB that we newly proposed were independently associated with both positive *M. tuberculosis* cultures (aOR, 2.30; 95% CI, 1.48-3.58) and positive AFB smears (aOR, 1.85; 95% CI, 1.05-3.25; Table 2).

When all sputum specimens (n = 3) showed a WBC count of ≥ 25 cells/LPF, the percentages of positive *M. tuberculosis* cultures and positive AFB smears were both 48.1%. However, when all specimens showed a WBC count of < 25 cells/LPF, only 30.0% of the specimens had positive *M. tuberculosis* cultures and 30.0% of the specimens had positive AFB smears (Table 3).

In a sensitivity analysis using 91 specimens from 31 patients

Table 2. Multivariate logistic regression model for the prediction of AFB smear and tuberculosis culture positivity

Specimens	No.	Positive AFB smear		Positive TB culture	
		No. (%)	aOR§ (95% CI)	No. (%)	aOR§ (95% CI)
Sputum gram stain grade					
Grade 1 (WBC < 10/LPF, EPC ≥ 25/LPF)	43	9 (20.9)	1	7 (16.3)	1
Grade 2 (WBC 10-25/LPF, EPC ≥ 25/LPF)	26	4 (15.4)	0.72 (0.29-1.84)	7 (26.9)	1.48 (0.71-3.08)
Grade 3 (WBC ≥ 25/LPF, EPC ≥ 25/LPF)	44	15 (34.1)	1.33 (0.60-2.95)	19 (43.2)	2.84 (1.43-5.64)
Grade 4 (WBC ≥ 25/LPF, EPC 10-25/LPF)	26	6 (23.1)	1.22 (0.52-2.88)	11 (42.3)	2.98 (1.47-6.06)
Grade 5 (WBC ≥ 25/LPF, EPC < 10/LPF)	57	21 (36.8)	1.52 (0.67-3.41)	22 (38.6)	3.38 (1.67-6.87)
Grade 6 (WBC < 10/LPF, EPC < 10/LPF)	32	3 (9.4)	0.48 (0.19-1.23)	11 (34.4)	1.67 (0.86-3.26)
Sputum criteria for bacterial pneumonia					
Unacceptable specimen*	145	31 (21.4)	1	45 (31.0)	1
Acceptable specimen†	83	27 (32.5)	1.43 (0.87-2.37)	33 (39.8)	1.66 (1.11-2.49)
Sputum criteria for TB (proposed)					
WBC < 25/LPF‡	101	16 (15.8)	1	26 (25.7)	1
WBC ≥ 25/LPF‖	127	42 (33.1)	1.85 (1.05-3.25)	52 (40.9)	2.30 (1.48-3.58)

*Unacceptable specimen (Gram stain grade 1, 2, 3, and 6); †Acceptable specimen (Gram stain grade 4 and 5); ‡WBC < 25/LPF (Gram stain grade 1, 2, and 6); §adjusted for age, sex, and education group; ‖WBC ≥ 25/LPF (Gram stain grade 3,4, and 5). aOR, adjusted odds ratio; CI, confidence interval; AFB, acid-fast bacilli; TB, tuberculosis; WBC, white blood cell; LPF, low-power field; EPC, squamous epithelial cells.

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Table 3. Positive rate of acid-fast bacilli smears and tuberculosis cultures in patients with ≥ 3 consecutive sputum specimens according to the WBC count

WBC counts	Patients n	Positive AFB smear n (%)	Positive TB culture n (%)
All specimens with WBC ≥ 25/LPF	27	13 (48.1)	13 (48.1)
1 or 2 specimens with WBC ≥ 25/LPF	25	5 (20.0)	9 (36.0)
All specimens with WBC < 25/LPF	20	6 (30.0)	6 (30.0)

WBC, white blood cell; LPF, low-power field.

Table 4. Sensitivity analysis for AFB smear and TB culture positivity in microscopically confirmed patients

Specimens	n	Positive AFB smear		Positive TB culture	
		n (%)	aOR§ (95% CI)	n (%)	aOR§ (95% CI)
Sputum criteria for bacterial pneumonia					
Unacceptable specimen*	56	27 (48.2)	1	45 (80.4)	1
Acceptable specimen†	35	22 (62.9)	1.9 (0.79-4.56)	33 (94.3)	4.6(0.93-22.97)
Sputum criteria for TB (proposed)					
WBC < 25/LPF‡	35	13 (37.1)	1	26 (74.3)	1
WBC ≥ 25/LPF‖	56	36 (64.3)	3.46 (1.38-8.69)	52 (92.9)	5.30 (1.40-20.4)

*Unacceptable specimen (Gram stain grade 1, 2, 3, and 6); †Acceptable specimen (Gram stain grade 4 and 5); ‡WBC < 25/LPF (Gram stain grade 1, 2, and 6); §adjusted for age, sex, and education group; ‖WBC ≥ 25/LPF (Gram stain grade 3,4, and 5). aOR, adjusted odds ratio; CI, confidence interval; AFB, acid-fast bacilli; TB, tuberculosis; WBC, white blood cell; LPF, low-power field; EPC, squamous epithelial cells.

with microscopically confirmed TB (Table 4), the specimens with WBC > 25 showed statistically higher positive AFB-stain (64.3% vs. 37.1% in the group with WBC > 25/LPF, $P = 0.012$) and *M. tuberculosis* culture rates (92.9% vs. 74.3%, $P = 0.014$). The multiple regression model adjusted for age, gender, and education showed similar results. However, sputum criteria for bacterial pneumonia failed to show a statistical significance in this sensitivity analysis (Table 4). Specimen with a WBC count of ≥ 25 cells/LPF was not significantly associated with the extent of lesion and the presence of cavity in chest X-ray.

DISCUSSION

Sputum leukocytosis was a good indicator for positive *M. tuberculosis* cultures and positive AFB smears in patents with suspected pulmonary TB. We proposed a new sputum criterion for TB, defined as a sputum specimen with a WBC count of ≥ 25 cells/LPF, which was a good predictor of positive *M. tuberculosis* cultures and positive AFB smears; the criteria for bacterial pneumonia were also able to predict positive *M. tuberculosis* culture.

True pathogens and oropharyngeal (i.e., saliva) contamination should be differentiated when diagnosing bacterial pneumonia. Therefore, an acceptable sputum specimen with a high WBC and low epithelial cell count would be a good indicator of sputum quality for diagnosing bacterial pneumonia (1).

We identified an association between sputum leukocytosis and mycobacterial positivity, which corresponds with findings from a previous retrospective study. McCarter et al. (10) evaluated 665 sputum specimens and showed that a total of 51 (7.0%) primary smears and 121 (16.7%) bacterial cultures were positive for mycobacteria. Approximately 92.2% of the positive smears and 90.1% of the positive cultures from these specimens contained neutrophils. Although we did not analyze the sputum specimens according to WBC counts, all specimens with positive AFB staining (n = 58) and positive *M. tuberculosis* culture (n = 77) had a WBC count of > 10 cells/LPF. No specimens with a positive AFB stain and positive *M. tuberculosis* culture had a WBC count of < 10 cells/LPF.

Purulent sputum is associated with more neutrophils, a higher degree of inflammation, and bacterial isolation (11-13). Furthermore, the gross appearance of sputum in patients with suspected TB may contribute to the increase in smear positivity (14-17). Therefore, sputum specimens with higher WBC counts might reflect severe inflammation and greater mycobacterial counts.

According to our results, sputum leukocytosis might be an effective indicator for evaluating sputum acceptability for pulmonary TB diagnosis. We cautiously suggest that the instructions for patients indicate "purulent sputum" instead of "no saliva" (3).

However, this study does not mean that sputum specimens with WBC < 25/LPF should be discarded to diagnose tuberculosis. *M. tuberculosis* in sputum is rarely contaminated (13). In a study by Isaac-Renton et al. (4), salivary specimens yielded 30.9% of the total 42 *M. tuberculosis* isolates and 19% of the 21 positive smear and culture specimens. The concept of 'acceptability' in this study is 'an indicator related to positive tuberculosis' and sputum WBC count could be the indicator. It is not easy to perform Gram staining to evaluate acceptability of sputum for TB in clinical practice. Therefore, further investigations regarding this are needed.

In conclusion, a WBC count of > 25 cells/LPF with a positive Gram stain could be an indicator for appropriate sputum specimens for diagnosing pulmonary TB and further large study is needed.

DISCLOSURE

The authors declare that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

AUTHOR CONTRIBUTION

Conception and design: Lee YJ, Lee CH, Kim DK, Chung HS. Acquisition of data, drafting, revision: Lee YJ, Lee CH, Roh EY, Yoon JH. Interpretation of data: Kim DK, Chung HS. Approval of manuscript and submission: All authors.

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