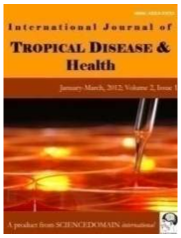

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Factors Affecting Discordance between Sputum Smear and Culture Conversion Rates In Newly Diagnosed Pulmonary Tuberculosis: A Retrospective Study in Developing Country

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Authors' contributions

This work was carried out in collaboration between all authors. Author DD wrote the protocol and the first draft of the manuscript. Authors VD and VT performed the statistical analysis. Authors KP, PJ, PC, YK, CB and CC provided comments for the manuscript. Author PP developed study conception, study design, organized as well as supervised the study, review and comment on manuscript for publication. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To assess the performance of sputum AFB smear for monitoring treatment response and outcome of anti-tuberculous drugs among newly diagnosed smear positive

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pulmonary TB patients.
Study Design: This study was conducted prospectively among newly diagnosed smear positive pulmonary TB patients.
Place and Duration of Study: Queen Savang Vadhana Memorial Hospital and Chonburi Regional Hospital, Chonburi province, Thailand during April 2010 and July 2012.
Methodology: Sputum AFB smear, culture and drug susceptibility test were performed at the time of diagnosis, the second and the fifth month of treatment. Baseline characteristic, clinical and laboratory parameters, treatment regimens and adverse events were recorded. Descriptive statistics and multiple logistic regression analysis were applied as appropriate. The performance of sputum AFB smear for monitoring treatment response and outcome of anti-tuberculous drugs was done using culture as the gold standard.
Results: Of 297 eligible pulmonary TB cases, majorities were male (72.4%) with median age of 39 years, illiterate to low educated (52.6%) and earning low income (77.5%). Cough was the most common symptom (91.2%) and cavity was present in 31.1%. At the second month, 17.0% of patients had discordance between sputum AFB smear and culture. High bacilli load (adjusted OR=2.38, CI=1.09-5.18), and hearing alteration (adjusted OR=10.98, CI=1.79-67.28) were significant predictors. Hypoalbuminemia was significantly more severe in patients with false positive AFB smear (*P*=.04). Sensitivity and specificity for AFB smear were 44.7% and 89.6% at the second month and 57.1% and 97.5% at the fifth month, respectively. MDR-TB was diagnosed in 1.0% and success rate was 77.1%.
Conclusions: Baseline AFB smear ≥ 2+ and hypoalbuminemia as well as adverse events during intensive phase are strongly recommended as the criteria to prioritize culture and DST for new smear positive pulmonary TB patients with positive AFB smear at the second and the third month of treatment in developing countries.

Keywords: AFB smear; sputum culture; microbiological outcome; primary drug resistance; discordance.

1. INTRODUCTION

Tuberculosis (TB) is a common cause of death worldwide according to world health organization (WHO) report in 2012 [1]. In 2011, 4.6 million people were new cases of pulmonary TB of which approximately one half of them had sputum smear positive for acid-fast bacilli (AFB) [1]. Sputum smear for AFB and mycobacterium culture are used for diagnosis of pulmonary TB and treatment monitoring. Mycobacterium culture is more sensitive than sputum smear microscopy as describe elsewhere [2,3] and it remains the gold standard for diagnosis of tuberculosis [3,4]. Previous reports showed that sensitivity and specificity of sputum AFB smear before treatment were 50–70% and 92%, respectively [2,5]. At the second month of treatment, sensitivity and specificity of sputum smear were 71% and 70%, respectively [6] and false positive AFB smear was reported to be 44-51% [6,7]. The primary multidrug resistant TB (MDR-TB) was estimated globally to be 3.6% in new pulmonary TB cases in 2011[1]. In Thailand, WHO report 2012 showed that the estimated MDR-TB was approximately 2% in new pulmonary TB cases [1]. The diagnosis of primary MDR-TB depend on mycobacterium culture and drug susceptibility test (DST) result which are rarely performed initially in high prevalence low income-countries due to time-consumption, cost and availability of facilities. In 2011, there were 4.7 laboratories with mycobacterium culture per 5 million Thai populations [1]. Treatment monitoring for pulmonary TB in Thailand is generally relied on sputum smear while mycobacterium culture is not a routine practice especially, in newly diagnosed TB patients. Treatment success in

Thailand gradually increased from 64% in 1995 and recently, it was able to reach WHO goal of 85% in 2009 and 2010 [1]. On the other hand, relapse cases keep increasing since 2000 while success rate among these cases declined from 68% in 2009 to 66% in 2010 [1]. These may indicate drug resistance as well as treatment failure in new pulmonary TB patients. Furthermore, the new target success rate in global plan to stop TB 2011-2015 is 90% [1]. Therefore, Thailand national TB control program need to revise the strategies to overcome possible obstacles and accomplish this new target.

Effective monitoring for treatment response in newly diagnosed TB patients can contribute to success of TB control. However, there were only few studies using sputum culture for monitoring treatment response as well as assessing outcome in limited resource countries. Therefore, a prospective study was conducted in order to determine sensitivity of sputum AFB smear for monitoring treatment response and microbiological outcome of anti-tuberculous treatment in newly diagnosed TB patients in limited resource setting in Thailand.

2. METHODOLOGY

2.1 Study Design and Data Collection

A prospective observational study was conducted at Queen Savang Vadhana Memorial Hospital and Chonburi Regional Hospital, Chonburi province located in eastern part of Thailand during April 2010 and July 2012. The inclusion criteria were: (1) patients with aged ≥14 years; (2) having ≥2 pulmonary or constitutional symptoms including cough ≥2 weeks, dyspnea, chest pain or discomfort, hemoptysis, fever, fatigue, malaise, weight loss and night sweat; (3) defined as pulmonary TB due to presence of abnormal chest radiography including reticular, interstitial, nodular infiltrate, or cavity and having 1 or more sputum smear positive for AFB according to the WHO definition 2012 [1] and (4) defined as new cases of TB according to the WHO definition 2012 [1].

Baseline characteristic, clinical and laboratory parameters as well as treatment regimens were recorded in a pre-defined case record form. Adverse events and treatment adherence were evaluated monthly during follow up period. At the second month, treatment response of anti-tuberculous drugs was evaluated by sputum smear for AFB and culture for mycobacterium. Treatment outcome was evaluated by sputum smear for AFB or culture for mycobacterium and chest radiography at the fifth month and by sputum smear for AFB at the end of treatment.

2.2 Laboratory Investigations and Sputum Examinations

Complete blood count, blood chemistries and HIV testing were done at the time of diagnosis. Sputum examinations were performed by sputum smear for AFB and culture for mycobacterium prior to treatment, at the second and the fifth month of treatment. Only at the end of treatment, sputum smear for AFB was performed. Sputum smear for AFB was performed using the Kinyoun method [8] at the treatment hospitals and AFB smear was graded according to 1998 WHO Laboratory Services In Tuberculosis Control [9]. At each time point, only the highest AFB positive specimen of each patient with sputum AFB positive was transferred under temperature 2- 8ºC to the national TB reference laboratory center in Thailand for mycobacterium culture with its DST otherwise only the best quality AFB negative specimen was transferred. All specimens were treated with standard digestion and decontamination with NaOH-NALC-sodium citrate solution as well as concentration prior to mycobacterium culture as described elsewhere [8]. Mycobacterium cultures were performed by automated liquid media culture systems (MGIT 960, BACTEC, USA) as the gold standard and conventional method using Löwenstein–Jensen solid media. The culture positive specimens were reported according to 1998 WHO Laboratory Services In Tuberculosis Control [10] and MGIT TM Procedure Manual [8].

2.3 Definitions

- Household TB contact: contact with the TB index cases in the same living area.
- Community TB contact: contact with the TB index cases such as friends, colleagues or neighbor.
- Intensive phase: Standard TB treatment phase consists of isoniazid (H), rifampicin (R), pyrazimanide (Z) and ethambutal (E) for 2 months [11].
- Continuation phase: Standard TB treatment phase consists of isoniazid (H) and rifampicin (R) for 4 months [11].
- Successful outcome: sum of patients with cure and treatment completed [11].
- Cure: A patient whose sputum smear or culture was positive at the beginning of the treatment but who was smear or culture negative in the last month of treatment and on at least one previous occasion [11].
- Treatment completed: A patient who completed treatment but who does not have a negative sputum smear or culture result in the last month of treatment and on at least one previous occasion [11].
- Risk of alcohol consumption: criteria based on a single drinking day [12]:

    - Low Risk; males: 1 to 40 g, females: 1 to 20 g
    - Medium Risk; males: 41 to 60 g, females: 21 to 40 g
    - High Risk; males: 61 to 100 g, females: 41 to 60 g
    - Very High Risk; males: ≥101 g, females: ≥ 61 g

- Adherence to treatment: the patient's history of therapeutic drug taking exactly corresponding with the prescribed treatment [13]

2.4 Sample Size Calculation

The sample size calculation is stated as below:

$$n = \frac{z^2 \times p \times (1-p)}{d^2}$$

whereas:

n = sample size, z = 1.96 (α = 0.05), p = the success rate of TB treatment, d = error allowance 5%

$$n = \frac{1.96^2 \times 0.77 \times 0.23}{0.05^2}$$

The sample size was estimated based on the Thailand national TB program monitoring and evaluation report 2006-2007 showing 77% cure rate of new AFB smear positive patients [14] with a 95% confidence interval (CI) and the precision to be within 5% of the true value. A required sample size of at least 272 pulmonary TB patients was enrolled in our study.

2.5 Statistical Analysis

Data were analyzed using the statistical package SPSS for Windows version 18.0 (SPSS, Chicago, IL). Categorical variables were summarized as frequencies (percentages) and $\chi 2$ test or the Fisher's exact test was applied where appropriate for two group comparison. Numerical variables were tested for normality using the Shapiro Wilk test, if non-normal distributed data, summarized as median (inter-quartile range; IQR) and compared using Mann-Whitney U test for two group comparison. Logistic regression analysis was used to determine the factor affecting discordance between sputum AFB smear and mycobacterium culture at the second month of treatment. Univariate logistic regression analysis was performed to establish the association of each factor with the discordance between two methods and multivariate logistic regression analysis was strictly applied to variables with $P \leq 0.2$ in univariate analysis to identify the factor affecting discordance between two methods. The accuracy of sputum AFB smear including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were defined using mycobacterium culture as gold standard and expressed as a proportion with exact 95% CI.

3. RESULTS

3.1 Demographic and Clinical Characteristics of Newly Diagnosed Pulmonary TB Patients

A total of 297 newly diagnosed pulmonary TB patients were eligible for the study. Median age (IQR) was 39 years (29.5-50.0). Majorities were as follows; males (215/297, 72.4%), low educated (154/274, 56.2%), laborers (158/296, 53.4%), low income (217/280, 77.5%), active smokers (84/294, 28.6%), very high risk of alcohol consumption (76/115, 66.1%) and household TB contact (106/297, 35.7%) (Table1). Diabetes mellitus (47/296, 15.9%) and HIV infection (34/296, 11.9%) were the major co-morbidities. Extrapulmonary TB was concomitantly diagnosed in 5.1% of patients (15/292).

Productive cough (246/294, 83.7%) was the most common symptom. Other common symptoms were as follows; fever (210/295, 71.2%), shortness of breath (195/295, 66.1%), fatigue (160/294, 54.4%) and chest pain (154/294, 52.4%). Median body weight reduction (IQR) was 8.6% (3.9-14.4%). Cavity was found in 31.1% of patients (80/256). There was slightly low level of hemoglobin (median 11.5 g/dl, IQR 9.9-13.1), hematocrit (median 34.6%, IQR 30.6-39.4), sodium (median 134.0 mmol/l, IQR 130.0-137.1), chloride (median 99.0 mmol/l, IQR 96.0-103.0) and albumin level (median 3.3 g/dl, IQR 2.8-3.7).

3.2 Sputum AFB Smear and Culture for *M. Tuberculosis*

Of 297 AFB smear positive patients, 278 (93.6%) patients had culture positive for *M. tuberculosis* at the time of diagnosis. Of 266 patients remaining at second month, culture could not be obtained from 7 patients because of contamination while 38 patients were culture positive. Furthermore of 249 patients remaining at fifth month, culture could not be obtained from one patient because of contamination while 7 patients were culture positive.

The sensitivity, specificity, PPV and NPV for AFB smear of the second month were 44.7%, 89.6%, 42.5% and 90.4%, respectively (Table 2). Of 38 patients with culture positive for *M. tuberculosis* during the second month of treatment, 17 patients (44.7%) were AFB smear positive and 21 patients (55.3%) were AFB smear negative, of which 2 patients remained culture positive at the fifth month of treatment. At the fifth month of treatment, the sensitivity, specificity, PPV and NPV for AFB smear were 57.1%, 97.5%, 40.0% and 98.7%, respectively (Table 2). During treatment, PPV of AFB smear was less than 50% indicating that more than half of AFB smear positive patients had non-viable bacilli.

Table 1. Demographic characteristics of newly diagnosed pulmonary TB patients

Characteristics	n	no (%)/median (IQR)
Age (years)	297	39.0 (29.5-50.0)
Gender: male	297	215 (72.4)
Married	296	179 (60.5)
Illiterate or primary school attainment	274	154 (56.2)
Occupation: laborer	296	158 (53.4)
Income (USD/month) ≤ 300	280	217 (77.5)
Smoking status:	294	
Smoking duration (months)	180	21.0 (12.2-30.8)
Smoking pack year	177	13.0 (7.0-24.9)
Very high risk of alcoholic consumption[a]	115	76 (66.1)
Duration of alcoholic consumption (months)	89	228.0 (132.0-318.0)
Drug abuse	291	61 (21.0)
Duration of drug abuse (months)	43	24.0 (12.0-60.0)
Diabetes mellitus	296	47 (15.9)
HIV positive	296	34 (11.9)
History of TB contact	297	
BCG scar	284	223 (78.5)
Extrapulmonary TB	292	15 (5.1)
Duration of follow up (months)	203	9.6 (8.9-11.4)

[a] *International guide for monitoring alcohol consumption and related harm [12];*
IQR, interquartile range

Table 2. Sensitivities and specificity of sputum AFB smear using culture as the gold standard

Mycobacterium culture	Second month of treatment			Fifth month of treatment		
	Sputum AFB microscopy		Total	Sputum AFB microscopy		Total
	Positive	Negative		Positive	Negative	
Positive	17	21	38	4	3	7
Negative	23	198	221	6	235	241
Total	40	219	259	10	238	248
% (95% CI)						
Sensitivity	44.7 (28.6-61.7)			57.1 (18.8-89.6)		
Specificity	89.6 (84.8-93.3)			97.5 (94.7-99.1)		
Positive Predictive Value	42.5 (27.1-59.1)			40.0 (12.4-73.6)		
Negative Predictive Value	90.4 (85.7-94.0)			98.7 (96.4-99.7)		
Accuracy	83.0 (78.4-87.6)			96.4 (94.0-98.8)		

CI, confidence interval.

3.3 Discordance between Sputum AFB Smear and Culture during Treatment

There were 259 patients having complete result of sputum AFB smear and culture at second month of treatment. Of which, 44 patients (17.0%) had discordance between sputum AFB smear and culture. Patients with and without discordance were compared and the predictors were also demonstrated (Table 3). Proportion of patients having cough (*P*=.03) and sputum AFB smear ≥ 2+ (*P*=.01) at baseline and hearing alteration during intensive phase (*P*=.003) were significantly higher in patients with discordance. Consequently, duration of treatment in intensive phase was significantly prolonged in patients with discordance (*P*=.01). However, proportion of patients with sputum culture positive at the fifth month of treatment and successful outcome were similar between these two groups. Logistic regression analysis showed that baseline sputum AFB smear ≥ 2+ (adjusted OR=2.38, CI=1.09-5.18) and hearing alteration during intensive phase (adjusted OR=10.98, CI=1.79-67.28) were the significant predictors for discordance between sputum AFB smear and culture at second month of treatment.

Of 44 patients (17.0%) with discordance between sputum AFB smear and culture, 21 patients (8.1%) had false negative sputum smear and 23 patients (8.9%) had false positive sputum smear. All characteristics and parameters of patients with false negative were similar to patients with true positive and negative AFB smear (Table 4). On the other hand, patients with false positive AFB smear had significantly higher proportion of patients with chest pain (*P*=.02) and AFB smear ≥ 2+ (*P*=.001) but lower level of baseline albumin (*P*=.04) than patients with true negative sputum AFB smear (Table 5). Furthermore, they had significantly higher proportion of patients with chest pain (*P* =.04) and higher level of albumin at the second month (*P* =.02) than patients with true positive sputum AFB smear (Table 5).

At the fifth month of treatment, 10 of 248 patients (4.0%) had discordance between sputum AFB smear and culture. All parameters were similar between patients with and without discordance except proportion of patients with positive AFB smear at the second month was significantly higher in discordant group (5/10, 50.0% VS 35/238, 14.71%; *P*=.04).

3.4 Drug Susceptibility Testing and Treatment Outcome

Primary drug resistance was found in 28 of 290 patients (9.7%), of which monodrug resistance was found in 17 (5.9%) patients; 9 patients (3.1%) with isoniazid resistance and 8 patients (2.8%) with streptomycin resistance. Of 9 patients with isoniazid resistance, 6 patients had AFB smear and culture negative at the second month while 2 patients had culture negative at the fifth month and 1 patient were transferred out. Of 8 patients with streptomycin resistance, 6 patients had AFB smear and culture negative at the second month and 2 patients were transferred out. In addition, there were 11 patients with primary polydrug resistances; 9 patients with both isoniazid and streptomycin resistance and 2 patients with MDR-TB. Among patients with both isoniazid and streptomycin resistance, 7 of them had sputum smear and culture negative at the second month but one had sputum smear and culture reversion at the fifth month. MDR-TB was diagnosed in 3 patients; 2 patients with primary MDR-TB and 1 patient with acquired MDR-TB at the fifth month of treatment.

Table 3. Factors affecting sputum AFB smear and culture discordance at second month of treatment

Characteristics	n	Sputum AFB smear and culture at 2 months of treatment								
		Discordance		Concordance		*P* value[a]	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
		n	n (%)/Median (IQR)	n	n (%)/ Median (IQR)					
At baseline										
Age (years)	259	44	43.5 (31.2-53.0)	215	38.0 (28.0-48.0)	.09	0.98 (0.96-1.00)	.12	0.98 (0.96-1.00)	.19
Cough	257	43	43 (100.0)	214	193 (90.2)	*.03*	2.19 (0.74-6.48)	.16	1.57 (0.50-4.96)	.44
Sputum AFB ≥ 2 +	259	44	29 (65.9)	215	93 (43.3)	*.01*	2.54 (1.29-4.99)	*.007*	2.38 (1.09-5.18)	*.03*
Intensive phase										
Hearing alteration	225	42	5 (11.9)	183	2 (1.1%)	*.003*	12.11 (2.26-64.73)	*.004*	10.98 (1.79-67.28)	*.01*
Outcome										
Sputum culture positive at 5 months	242	40	2 (5.0)	202	5 (2.5)	.33	3.12 (0.72-13.77)	.13	2.52 (0.34-18.51)	.36
Successful outcome	228	37	33 (89.2)	191	181 (94.8)	.25	0.47 (0.20-1.10)	.08	0.57 (0.14-2.38)	.44

[a] *chi squared test; IQR, interquartile range; OR, odds ratio; CI, confidence interval.*

Table 4. Demographic characteristics, clinical and laboratory parameters of patients with false negative, true positive and negative sputum AFB smear at the second month of treatment

Characteristics	False negative		True positive		*P* value[b]	True negative		*P* value[c]
	n	n (%)/Median	n	n (%)/Median		n	n (%)/Median	
Demography								
Male	21	16 (76.2)	17	14 (82.4)	.71	198	136 (68.7)	.65
Age (years)	21	37.0 (30.5-53.0)	17	39.0 (35.0-55.0)	.54	198	37.0 (28.0-47.2)	.42
Illiteracy	19	14 (73.7)	16	9 (56.3)	.47	184	99 (53.8)	.16
High to very high risk	12	10 (83.3)	5	5 (100.0)	1.00	73	63 (86.3)	.68
History of TB contact	21	11 (52.4)	17	9 (52.9)	1.00	198	94 (47.5)	.84
Family history of TB	21	9 (42.9)	17	7 (41.2)	1.00	198	72 (36.4)	.73
HIV	21	1 (4.8)	16	2 (12.5)	.57	192	24 (12.5)	.48
DM	21	2 (9.5)	17	3 (17.6)	.64	198	31 (15.7)	.75
Baseline								
Hemoptysis	20	4 (20.0)	17	6 (35.3)	.46	197	67 (34.0)	.31
Chest pain	20	11 (55.0)	17	7 (41.2)	.61	197	100 (50.8)	.90
Hemoglobin (g/dl)	14	11.6 (10.0-13.0)	11	10.4 (7.9-12.5)	.24	128	11.6 (10.2-13.2)	.95
Hematocrit (%)	14	34.3 (30.4-38.8)	11	31.6 (24.6-37.9)	.27	128	35.0 (31.0-39.6)	.85
Platelet count ($x10^3/mm^3$)	14	434.0 (357.0-586.8)	11	591.0 (418.0-	.22	128	397.0 (289.5-481.8)	.15
Cavity	18	6 (33.3)	15	8 (53.3)	.42	169	47 (27.8)	.83
Aspatate	16	34.0 (21.2-65.7)	11	21.0 (18.0-34.0)	.10	144	26.0 (19.2-43.8)	.21
Alanine aminotransferase	16	28.5 (15.0-55.2)	11	18.0 (9.0-29.0)	.21	145	21.0 (13.0-36.0)	.32
Alkaline phosphatase	14	119.5 (77.8-206.5)	11	101.0 (83.0-212.0)	.89	142	92.0 (70.0-137.8)	.20
Albumin (g/dl)	14	3.4 (3.0-3.9)	11	3.1 (2.9-3.4)	.27	142	3.4 (3.0-3.7)	.94
Sputum AFB≥ 2+	21	11 (52.4)	17	14 (82.4)	.11	198	79 (39.9)	.38
Sputum culture positive	20	2 (10.0)	17	0	.49	185	5 (2.7)	.14
INH resistance	21	1 (4.8)	17	1 (5.9)	1.00	198	6 (3.0)	.51
MDR	21	1 (4.8)	17	0	1.00	198	0	.10
At second month								
Aspatate	5	41.0 (22.0-56.0)	4	25.5 (18.5-35.5)	.19	47	26.0 (21.0-36.0)	.30
Alanine aminotransferase	5	17.0 (10.0-27.0)	4	16.5 (8.2-26.2)	.56	47	18.0 (13.0-30.0)	.57
Alkaline phosphatase	5	103.0 (73.0-223.5)	4	100.5 (88.0-110.8)	.73	45	79.0 (70.5-101.0)	.15
Albumin (g/dl)	5	3.7 (2.2-4.4)	4	3.0 (2.7-3.4)	.41	41	3.8 (3.3-4.1)	.68
Cavity	9	4 (44.4)	6	3 (50.0)	1.00	91	22 (24.2)	.23
INH resistance	19	1 (5.3)	17	2 (11.8)	.59	198	0	.09
MDR	19	1 (5.3)	17	0	1.00	198	0	.09

Continued Table 4 ……………

Treatment and outcome								
Duration	21	60.0 (55.0-71.5)	17	92.0 (56.5-99.0)	.04	197	62.0 (54.0-71.0)	.99
Adherence	21	1 (4.8)	17	1 (5.9)	1.00	198	165 (83.3)	.21
Successful outcome	19	16 (84.20	16	15 (93.8)	.61	185	176 (95.1)	.09

[a] International guide for monitoring alcohol consumption and related harm [12]; [b] false negative AFB smear VS true positive AFB smear; [c] false negative AFB smear VS true negative AFB smear; IQR, interquartile range

Table 5. Demographic characteristics, clinical and laboratory parameters of patients with false positive, true negative and positive sputum AFB smear at the second month of treatment

Characteristics	False positive		True negative		***P* value**[b]	True positive		***P* value**[c]
	n	**n (%)/Median**	**n**	**n (%)/Median**		**n**	**n (%)/Median**	
Demography								
Male	23	17 (73.9)	198	136 (68.7)	.78	17	14 (82.4)	.71
Age (years)	23	46.0 (35.0-54.0)	198	37.0 (28.0-47.2)	.05	17	39.0 (35.0-55.0)	.73
Illiteracy	22	13 (59.1)	184	99 (53.8)	.81	16	9 (56.3)	1.00
High to very high risk of	9	6 (66.7)	73	63 (86.3)	.15	5	5 (100.0)	.26
History of TB contact	23	9 (39.1)	198	94 (47.5)	.59	17	9 (52.9)	.59
Family history of TB	23	7 (30.4)	198	72 (36.4)	.74	17	7 (41.2)	.71
HIV	22	1 (4.5)	192	24 (12.5)	.48	16	2 (12.5)	.56
DM	23	4 (17.4)	198	31 (15.7)	.77	17	3 (17.6)	1.00
Baseline								
Hemoptysis	23	9 (39.1)	197	67 (34.0)	.80	17	6 (35.3)	1.00
Chest pain	23	18 (78.3)	197	100 (50.8)	*.02*	17	7 (41.2)	*.04*
Hemoglobin (g/dl)	10	9.8 (9.2-12.0)	128	11.6 (10.2-13.2)	.08	11	10.4 (7.9-12.5)	1.00
Hematocrit (%)	9	30.6 (28.3-36.7)	128	35.1 (31.0-39.6)	.18	11	31.6 (24.6-37.9)	.82
Platelet count ($x10^3/mm^3$)	10	413.5 (369.2-	128	397.0 (289.5-	.41	11	591.0 (418.0-	.06
Aspatate aminotransferase	9	28.0 (19.5-68.0)	144	26.0 (19.2-43.8)	.73	11	21.0 (18.0-34.0)	.41
Alanine aminotransferase	9	16.0 (10.5-49.5)	145	21.0 (13.0-36.0)	.88	11	18.0 (9.0-29.0)	.82
Alkaline phosphatase (U/l)	9	101.0 (63.5-185.0)	142	92.0 (70.0-137.8)	.77	11	101.0 (83.0-	.66
Albumin (g/dl)	9	2.7 (2.4-3.4)	142	3.4 (3.0-3.7)	*.04*	11	3.1 (2.9-3.4)	.30
Cavity	21	9 (42.9)	169	47 (27.8)	.24	15	8 (53.3)	.78
Sputum AFB ≥2+	23	18 (78.3)	198	79 (39.9)	*.001*	17	8 (47.1)	1.00
Sputum culture positive	20	0	185	5 (2.7)	1.00	17	0	-
INH resistance	23	0	198	6 (3.0)	1.00	17	1 (5.9)	.43
MDR	23	0	198	0	-	17	0	-

Continued Table 5 ....

At second month								
Aspatate aminotransferase	6	23.5 (19.0-36.2)	47	26.0 21.0-36.0)	.63	4	25.5 (18.5-35.5)	1.00
Alanine aminotransferase	6	17.0 (13.8-22.2)	47	18.0 (13.0-30.0)	.69	4	16.5 (8.25-26.25)	.76
Alkaline phosphatase (U/l)	6	73.5 (53.5-90.2)	45	79.0 (70.5-101.0)	.38	4	100.5 (88.0-	.11
Albumin (g/dl)	6	3.6 (3.5-4.1)	41	3.8 (3.3-4.1)	.96	4	3.0 (2.7-3.4)	*.02*
Cavity	5	0	91	22 (924.2)	.59	6	3 (50.0)	.18
INH resistance	23	0	198	0	-	17	2 (11.8)	.17
MDR	23	0	198	0	-	17	0	-
Treatment and outcome								
Duration	23	86.0 (63.0-107.0)	197	62.0 (54.0-71.0)	*>.001*	17	92.0 (56.5-99.0)	.94
Adherence	23	6 (26.1)	198	33 (16.7)	.26	17	16 (94.1)	.21
Successful outcome	20	19 (95.0)	185	176 (95.1)	1.00	16	15 (93.8)	1.00

[a] International guide for monitoring alcohol consumption and related harm [12]; [b] false positive AFB smear VS true negative AFB smear
[c] false positive AFB smear VS true positive AFB smear; IQR, interquartile range

Among HIV and non-HIV TB patients, sputum examinations before treatment (culture positive: 97.1% VS 93.2%, *P*=.71), at the second month (AFB positive: 10.7% VS 15.7%, *P*=.78; culture positive: 10.7% VS 15.2%, *P*=.78) and the fifth month of treatment (AFB positive: 8.0% VS 3.2%, *P*=.24; culture positive: 4.0% VS 3.2%, *P*=.59) were similar. However, incidence of primary drug resistance before treatment was significantly higher in HIV TB patients than those with non-HIV [7/33 (21.2%) VS 20/245 (8.2%), *P*=.03).

Among patients with primary drug resistance, 82.4% (14/17) of patients with monodrug resistances and 77.7% (7/9) of patients with drug resistances to both isoniazid and streptomycin completed treatment and cured of TB using standard treatment regimen. This was due to time consuming processes of culture and DST. When the results were reported, patients already completed intensive phase and started continuation phase. Most physicians continued standard regimen in patients with clinical improvement and AFB smear negative though primary drug resistance was reported. Success and failure rate as determined by sputum culture were 77.1% and 2.5% while success and failure rate as determined by sputum smear was 78.1% and 1.4%.

Of 7 patients with culture positive at the fifth month, 2 patients were MDR-TB and 3 patients were false negative AFB smear (Table 6). It was noted that 5 patients had culture reversion, of which 2 patients had diabetes and 1 patient had HIV positive.

Table 6. Sputum examination profiles of patients with sputum culture positive at fifth month of treatment

Participants	Day 0		Second month		Fifth month	
	AFB smear	**Culture**	**AFB smear**	**Culture**	**AFB smear**	**Culture**
1	+	+	-	-	-	+
2[a]	+	+	-	+	+	+
3	+	+	-	-	+	+
4	+	+	-	+	-	+
5[b]	+	+	-	-	+	+
6	+	+	-	-	+	+
7	+	+	-	-	-	+

Note + = positive result, - = negative result,
[a] MDR TB at the time of diagnosis, [b] MDR TB at fifth month of treatment

4. DISCUSSION

In this study, sensitivity of AFB smear for monitoring treatment response was low. Of newly diagnosed pulmonary TB patients, 17.0% showed discordance between sputum AFB smear and culture at the second month. The success rate was 77.1% while MDR-TB was found in 1.0% of patients.

The sensitivity and specificity of AFB smear at the second month were 44.7% and 89.6% which was different from previous report showing sensitivity ranging 71-77% and specificity ranging 61-70% due to retreated cases [6,7]. Positive AFB smear could be due to nonviable mycobacterium [9,15] so the intensive phase of treatment should not be extended according to WHO guideline 2010 [11]. Our study showed that AFB smear was less sensitive than mycobacterium culture for monitoring treatment response which was similar to other report [6]. Failure rate was nearly 2 times higher by sputum culture (7 patients, 2.5%) compared to sputum AFB smear (4 patients, 1.4%). Of 7 patients with culture positive at the fifth month, 5 patients had culture reversion and 3 patients had false negative AFB smear. These indicated that sputum culture at the fifth month of treatment was potentially important.

Discordance between sputum AFB smear and culture in this study were significantly influenced by bacilli load. High bacilli load potentially affected sputum smear and culture conversion [6,16-19]. At the end of intensive phase, sputum smear positive with culture conversion could be observed due to nonviable bacilli, NTM, artifacts or contaminants such as food, stain precipitates [11,15]. On the other hand, improper sputum collecting and poor quality of sputum specimen yielded too small amount of viable bacilli to produce sputum AFB positive while *M. tuberculosis* was isolated [2,15]. Extension of intensive phase was significant finding among patients with positive AFB smear at the end of intensive phase contrasting WHO recommendation [11]. Previously, WHO recommended extending intensive phase for one more month in patients with sputum smear positive at the end of intensive phase [20]. Since there were no concrete evidences showing that extension of intensive phase can reduce failure and relapse rate regarding supervised rifampicin for entire course of treatment [11]. Then the recommendations have been changed, those patients can continue with continuation phase with sputum AFB smear monitoring at the end of third month [11]. The latest WHO recommendation should be informed and encouraged in a real practice. In addition, the linkage among adverse events, older age group and treatment interruption were demonstrated [2,11,21]. Although only one adverse event was significantly different between patients with and without discordance of sputum AFB smear and culture at second month of treatment, it may indicate the importance of close adverse event monitoring in order to enhance treatment adherence and subsequently, sputum conversion at the end of intensive phase.

MDR-TB (1.0%) in this study was similar to estimated MDR-TB cases in Thailand (1.7%) [1]. However, previous report from northern part of Thailand showed 6.3% of MDR-TB in new AFB smear positive pulmonary TB patients which was higher than this study possibly due to high HIV burden in that setting [22]. Subgroup analysis showed that MDR-TB was diagnosed in approximately 3-9% of new pulmonary TB patients with HIV positive [22,23] which might be due to direct transmission of MDR-TB to HIV patients or drug malabsorption especially, rifampin [24]. It was supported by the previous report showing 12 time higher risk of MDR-TB in HIV positive patients [25].

Outcome of treatment in this study was similar among patients with and without discordance of sputum AFB smear and culture at the second month of treatment because sputum AFB smear as well as culture at the second month of treatment had low sensitivity to predict treatment outcome especially treatment failure and relapse [7,26]. However, patients with sputum smear positive at the second month of treatment are great source of concern because it reflects TB control program [11]. Although patients with sputum smear negative after treatment are non infectious [27], patients with false negative sputum smear were responsible for 13% of TB transmission [28]. This rate was obtained from fluorescence microscopy with higher sensitivity than conventional microscopy used in this study so more patients with false negative sputum smear as well as more TB transmission were possible. High bacilli load (sputum AFB ≥2+) was the risk factors of sputum smear positive at the second month of treatment [29] similar to characteristics of patients with AFB smear and culture discordance as well as false positive AFB smear in this study. In addition, chest pain and serum albumin were significantly associated with false positive AFB smear. These factors could be use as the signal of discordance between sputum AFB smear and culture requiring close monitoring for anti-tuberculous response. These patients could be the candidate for sputum culture in the limited resource setting.

There were some limitations, recall bias which is unavoidable as data obtaining by interview. The conventional microscopy generally used in low income countries is less sensitive than fluorescence microscopy [30]. These lead to more false negative sputum AFB smear. Further study should be done to examine cost effectiveness of Mycobacterium culture in these specific subgroups of newly diagnosed pulmonary TB patients in developing countries.

5. CONCLUSION

At the second month, the sensitivity, specificity, PPV and NPV for AFB smear were 44.7%, 89.6%, 42.5% and 90.4%, respectively. At the fifth month of treatment, they were 57.1%, 97.5%, 40.0% and 98.7%, respectively. Sputum AFB smear ≥ 2+ and hearing alteration were the significant predictors for discordance between sputum AFB smear and culture at second month of treatment. In addition, severe hypoalbuminemia was significantly observed in patients with false positive AFB smear. WHO recommendation for culture and DST in new smear positive pulmonary TB patients may not be applicable in developing countries due to cost and limited facilities. Therefore, baseline sputum AFB smear ≥ 2+ and hypoalbuminemia as well as adverse events during intensive phase are strongly recommended as the criteria to prioritize culture and DST for new smear positive pulmonary TB patients with positive AFB smear at the second and the third month of treatment in developing countries.

CONSENT

Written informed consent was obtained from all patients.

ETHICAL APPROVAL

The study was approved by the ethics committee of Faculty of Tropical Medicine, Mahidol University in Bangkok and Queen Savang Vadhana Memorial Hospital and Chonburi Regional Hospital in Chonburi province, Thailand.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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