

Role of Routine Bronchial Washing Culture for TB in Maharat Nakhon Ratchasima Hospital, Thailand

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Objective: To evaluate the role of routine bronchial washings (BW) culture for diagnostic sputum smear negative pulmonary case of tuberculosis (TB) at Maharat Nakhon Ratchasima Hospital.

Material and Method: A retrospective, observational study of performed bronchial washing (BW) specimens from 93 patients who met presumptive criteria of smear negative case of pulmonary TB was done. The culture of BW for TB was done by conventional technique. All of the BW specimens were not centrifuged before AFB stain. Pathological reports were included in the study. Statistical analysis was described as percentage.

Results: Ninety-three patients were included in the presented study. Eighty-seven patients had a diagnosis of smear-negative pulmonary case of TB (93.5%). The un-centrifuged BW smear for AFB was positive in 17 (18.3%) patients while conventional culture of BW for TB was positive in 34 (36.6%) patients. The MDRTB and NTM were positive in one (1.1%) and five (5.4%) patients respectively. The positive un-centrifuged BW smear for AFB in the MDRTB and NTM patients were 0 and 2/5 (40%) respectively. The pathological reported granulomatous disease was found in 18 (19.4%) patients. Five (5.4%) patients had all positive of any methods while 45 (48.4%) patients had all negative tests.

Conclusion: The routine culture BW for TB should be done in every case of presumptive diagnosis of sputum smear negative pulmonary case of TB. Furthermore, it can establish that MDRTB and NTM cannot be identified with only AFB stain.

Keywords: Routine bronchial washing, Tuberculosis mycobacterium, Bronchoscopic examination, Sputum smear negative

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Tuberculosis (TB) is important preventable and treatable cause of death. Thailand is one of the 22 high TB burden countries with 2011 estimates of about 67,676 new cases of tuberculosis of all forms (smear positive was 33,169, smear negative was 20,726, extra-pulmonary was 10,014 and no report of smear not done)⁽¹⁾.

By definition, Smear-negative pulmonary case of TB is a patient with pulmonary TB who does not meet the above criteria for smear-positive disease. Diagnostic criteria should include at least two AFB-negative sputum smear examination, radiographic abnormalities consistent with active pulmonary TB, no response to a course of broad-spectrum antibiotic (except in a patient for whom there is laboratory confirmation or strong clinical evidence of HIV

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infection), and a decision by a clinician to treat with a full course of anti-TB chemotherapy. A patient with positive culture but negative AFB sputum examination is also a smear-negative case of pulmonary TB⁽¹⁾.

Early detecting in patients with active TB is an important component of TB control. This is because the beginning of appropriate treatment renders these patients with non-infected and stop the chain of TB transmission. Retrospectively reviews⁽²⁻⁴⁾ from Western countries proved that routine bronchial washing culture for *Mycobacterium tuberculosis* (TB) was costly and not indicated in a low prevalence TB country. On the contrary, in the moderate or high prevalence TB country, studies⁽⁵⁻⁷⁾ showed the benefit.

Thailand is a high prevalence TB country and there are no typical features of abnormal CXR or Chest CT that specified for active pulmonary TB. Sputum AFB is very helpful to diagnosis of pulmonary tuberculosis but sputum smear examination for AFB can establish up to 50 to 60% of cases of pulmonary TB in a standard laboratory⁽⁸⁾.

Flexible bronchoscope examination is very helpful to get the sample from lung parenchyma. For example, BW for AFB stain and culture, tissue biopsy for pathological report. Nevertheless, the diagnostic yield of bronchoalveolar lavage for AFB stain in case of smear-negative pulmonary TB was previously revealed, in Thailand, at 7.5%⁽⁹⁾. Hence, BW culture for TB possibly play an important role for confirming the diagnosis, although the cost of TB culture was higher than others and needs at least two weeks for positive result. Moreover, it can demonstrate MDRTB and NTM. This is because the pulmonary TB, MDRTB, and NTM cannot establish by AFB stain solely.

The presented study aimed to establish the role of routine culture BW for TB in the presumptive diagnosis of smear-negative pulmonary case of TB at Maharat Nakhon Ratchasima Hospital.

Material and Method

The present retrospective study, approved by the institutional ethics committee, was conducted in the Division of pulmonary and critical care, Department of medicine, Maharat Nakhon Ratchasima Hospital, Thailand. All medical records of patients who had performed flexible bronchoscope examination by the authors between October 1, 2008 and September 31, 2011 were reviewed. Four hundred sixty one patients had abnormal CXR or CT-chest finding. Sputum smear negative status were subjected to diagnostic fiberoptic bronchoscope. Ninety-three patients had a presumptive diagnosis of smear negative in case of pulmonary TB.

Patients

Patients were included for presumptive diagnosis of smear negative pulmonary in case of TB if they were 1) over >18 years, 2) had clinical suspicion of Pulmonary TB (based on constitutional symptoms for example, fever, weight loss, cough with or without hemoptysis, loss of appetite, malaise etc.), 3) had either the persistence or no improvement of abnormal radiological (CXR or CT-chest) finding after antibiotic treatment, and 4) had three consecutive sputum samples were negative for AFB stain (spot, morning and spot). They were excluded if they were HIV seropositive.

Bronchoscope procedure

All flexible bronchoscope procedures were done under conscious sedation by the experienced pulmonologist. The intravenous pethidine 25 mg injection was done as a premedication. A local anesthesia with 10% lidocain spray was applied to the

oropharynx before the procedure about 15 minutes. The vocal cord, trachea, and main bronchus were surveyed and bronchial washing (BW) from the affected part were accomplished, localized lesion by radiology and directed observation.

The bronchial washing were acquired by instilling 10 ml of 0.9% isotonic saline (NSS) at room temperature via the flexible bronchoscopic channel directly through the affected segment, pursued by current suction and connector system. The procedure was repeated until 30 to 40 ml of aliquot was collected for all analysis. The aliquot was exposed to AFB stain and conventional culture for TB. The endobronchial biopsy was done after fluid specimen had been collected for all analysis.

Culture for TB

The culture was done by conventional method with drugs sensitivity. The culture was performed on the specimen after decontamination and concentration technique by the addition of 4% sodiumhydroxide followed by centrifugation at 3,500 rpm, about 15 minutes. The deposit from each specimen was inoculated into two Lowenstein-Jensen slopes and into paranitrobenzoic acid medium (PNB) for identified TB, MDRTB and non-tuberculosis mycobacterium (NTM). The cultures were incubated for eight weeks and read weekly for growth of *M. tuberculosis*. All cultures reported as positive contained a significant number of colonies. Drug sensitivity was done next step after growth of TB with absolute concentration method.

Bronchial washing (BW) for AFB stain

The BW fluid were collected during flexible bronchoscope and sent to direct smear without centrifugation.

Statistical analysis

The percentage of positive BW culture for TB was determined. The data was interpreted by descriptive statistics and expressed as number and percentages (%).

Results

The baseline characteristic of patients whose presumptive diagnosis were smear negative of pulmonary TB is shown in Table 1. These patients included 66 men and 27 women with mean ages were 58.2±13.8 and 58.1±17.4 respectively. All patients had cough (100%), the second clinical presentation was

Table 1. Baseline characteristics of patients whose presumptive diagnosis was smear negative of pulmonary TB (n = 93)

	n = 93
Sex (male/female)	66/27
Age (male/female, year ± SD)	58.2±13.8/58.1±17.4
Hemoptysis (yes/no)	17/76
Dyspnea (yes/no)	92/1
Cough (yes/no)	93/0
CXR (mass/no mass)	37/56
Endobronchial lesion (yes/no)	30/63

Table 2. The largest diameter of lung mass/nodule in bronchial washing (BW) culture was positive for TB

Diameter (cm)	Number of patients = 37 (%)
<2 cm	1 (2.7)
>2-3 cm	9 (24.3)
>3-7 cm	22 (59.5)
>7-12 cm	5 (13.5)

dyspnea (98.9%). Hemoptysis was found the least 17/93 (18.3%). Mass like lesion from CXR was found 37/93 (39.8%), nearly endobronchial lesion 30/93 (32.3%) from flexible bronchoscopic examination.

In 37 (39.8%) of the 93 patients had lung mass. The most common size of diameter of lung mass was range 3 to 7cm that was found in 59.5%. The largest diameter of lung mass in the presented study was 12 cm (Table 2).

The most pathological in the present study was negative finding (55.9%), the second was granulomatous disease (19.4%), and the third was inflammation (15.1%). Seven patients had co-incidence between pulmonary TB and lung cancer, 5/7 (71.42%) by culture proven and pathological reports of malignancy cell (Table 3).

Forty patients had positive BW culture (43%) specimens while 34 (36.6%), one (1.1%), and five (5.4%) respectively were classified to TB, MDRTB, and NTM. Seventeen (18.3%) were positive BW for AFB and included one with negative outcome for culture TB. In cases of the negative BW for AFB, there was 24 (25.8%) positive culture. In the presented study, the positive BW culture for TB was revealed double of the positive BW for AFB (Table 4).

Five (5.4%) patients were positive in every tests. However, 45 (48.4%) patients had nothing

from laboratory and pathological report. Either the positive bAFB test or granulomatous disease from pathological report was unspecified for diagnosis pulmonary TB. This may be because they were found in MDRTB and NTM (Table 5).

Discussion

Thailand has a high prevalence of TB but the BW culture for TB was not routinely found in many tertiary hospitals. The presented study explored the value of the routine BW culture for TB in cases of presumptive diagnosis smear negative pulmonary TB, who underwent flexible bronchoscopic examination at Maharat Nakhon Ratchasima Hospital.

1979 Kvale et al⁽³⁾, 1981 Jett et al⁽¹⁰⁾, and 1999 Rubin and Bafenkamp⁽¹¹⁾ retrospectively reviewed routine bronchial specimens for culture TB. It had been suggested that the culture of bronchoscopic aspirates for TB should not be obtained routinely because they were reviewed in low TB burden countries.

Table 3. Pathological finding in bronchial washing (BW) culture is positive for TB, MDRTB and NTM

Pathological finding	Number = 93 (%)
Granulomatous disease	18 (19.4)
Malignancy cell	7 (7.5)
Inflammation	14 (15.1)
Necrotic tissue	2 (2.1)
Negative finding	52 (55.9)

* Co-incidence of pulmonary TB and Lung cancer by culture proven, bAFB and other tests were 7 patients

Table 4. Correlation between BW culture TB and bAFB in patients whose presumptive diagnosis was smear negative pulmonary TB (n = 93)

	BW culture for TB/MDRTB/NTM		Totals (%)
	Positive (%)	Negative (%)	
bAFB positive	16 (17.2)	1 (1.1)	17 (18.3)
bAFB negative	24 (25.8)	52 (55.9)	76 (81.7)
Totals	40 (43.0)	53 (57.0)	93 (100)

bAFB = bronchial washing for acid fast bacilli (AFB) stain; BW = bronchial washing; TB = *Mycobacterium tuberculosis*; MDRTB = multi-drugs resistance TB; NTM = non Tuberculosis Mycobacterium

There was one patient who had positive culture for MDRTB and negative for bAFB.

There were five patients who had positive culture for NTM and three of them had negative for bAFB.

Table 5. Bronchial washing (BW) culture for TB/MDRTB/NTM, pathological report and BW for AFB in Smear-negative pulmonary case of TB

BW for culture TB/MDRTB/NTM	Granulomatous disease	bAFB	Number (%) n = 93
+	+	+	5 (5.4)
+	+	-	5 (5.4)
+	-	+	11 (11.8)
+	-	-	19 (20.4)
-	+	+	1 (1.1)
-	+	-	7 (7.5)
-	-	+	0
-	-	-	45 (48.4)

bAFB = bronchial washing for acid fast bacilli (AFB) stain; BW = bronchial washing; TB = *Mycobacterium tuberculosis*, MDRTB = multi-drugs resistance TB; NTM = non Tuberculosis Mycobacterium

+ = positive; - = negative

There was one patient who had positive culture for MDRTB and negative for bAFB.

There were five patients who had positive culture for NTB and three of them had negative for bAFB.

On the contrary, 1989 Ip et al⁽¹²⁾ had reported positive cultures for TB at 6.9% in patients who underwent routine bronchoscopic examination in Hong Kong. 1982 Sarkar et al⁽²⁾ revealed positive cultures for TB in 14% of patients who did not suspect pulmonary TB in India. 2007 Yang et al⁽⁷⁾ reported 3.7% of patients grew TB from BW routinely culture TB in Taiwan recently. 2007 Kim et al⁽¹³⁾ found positive cultures for TB 6.4% of patients in Korea. They had suggested that bronchial washings should be examined routinely for tuberculosis because of the high diagnostic yield.

2005 Shitrit et al⁽⁴⁾ retrospectively studied the utility of routine culture of bronchoscopy samples of lung masses for TB in the tertiary-care hospital in Israel, a region where TB is not endemic. Culture positive for TB was found in 0.6% (1/168) and they were all negative for bAFB. In contrast, the present study found BW culture was positive for TB in 39.8% of pulmonary mass (37/93) and 48.4% of pulmonary infiltration (45/93). Neither MDRTB nor NTM patients had pulmonary mass. The positive bAFB was 25.8% (8/31) in case of lung masses. The reason for high positive results was high prevalence TB in the presented study.

2005 Charoenratanakul et al⁽⁹⁾ reported positive Bronchoalveolar lavage (BAL) for AFB in

40 patients. Of those patients, 7.5% were suspected smear negative pulmonary TB (3/40), 15% of positive for culture TB (6/40), and 17.5% of pathologically revealed granuloma (7/40). Furthermore, 15% were of NTM patients were diagnosed (6/40). The present study had different outcomes. The most positive finding was TB culture 34/93 (36.6%), the second was granulomatous disease (19.4%), and five NTM patients were diagnosed (5.4%). The positive AFB stain was found the lowest (18.3%). The reasons might be from different specimens (BAL and BW), number of patients, radiology report, bronchoscopic findings, and laboratory technique. The present study had an incidence of lung mass nearby endobronchial lesion (39.8%, 32.3%) that might affect the results of bAFB and culture TB. The incidence of MDRTB in the presented study was correlated with data of WHO that it was not a high MDR-TB burden in Thailand.

The limitations of the presented study were retrospective study, the un-centrifuged BW specimens, and conventional technique for TB culture. The centrifuged BW might be helpful for positive AFB stain and culture TB. Other specific tests might be useful in cases of NTM because it usually is either delayed diagnosis or misdiagnosed due to extended time for final report of culture TB.

Conclusion

The routine culture of bronchial washing for TB in the tertiary hospital in high prevalence TB burden country was recommended in case of smear negative pulmonary TB because it was proved worthy, benefited and early detected NTM/MDRTB that seldom identified with AFB stain only. Co-incidence of TB and lung cancer possibly occurs. Bacteriological examination is needed to confirm the diagnosis as it was essential to plan the appropriate management. The cost of all culture, AFB stain, and pathology in the present study was 55,800 baht (600 baht/specimen), 5,580 baht (60 baht/specimen), and 18,600 baht (200 baht/specimen) respectively. Even though the cost of culture was higher than others, it was reasonable.

Potential conflicts of interest

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References

1. World Health Organization. Tuberculosis (TB) [Internet]. 2012 [cited 2012 Nov 10]. Available

- from: <http://who.int/tb/en/>
2. Sarkar SK, Sharma TN, Purohit SD, Gupta ML, Gupta PR. The diagnostic value of routine culture of bronchial washings in tuberculosis. *Br J Dis Chest* 1982; 76: 358-60.
 3. Kvale PA, Johnson MC, Wroblewski DA. Diagnosis of tuberculosis: routine cultures of bronchial washings are not indicated. *Chest* 1979; 76: 140-2.
 4. Shitrit D, Dekel S, Bar-Gil SA, Kramer MR. The role of routine culture for tuberculosis during bronchoscopy examination of lung masses. *Respiration* 2005; 72: 402-5.
 5. Shimao T. Tuberculosis prevalence survey in Japan. *Kekkaku* 2009; 84: 713-20.
 6. Niwa H, Tanahashi M, Kondo T, Ohsaki Y, Okada Y, Sato S, et al. Bronchoscopy in Japan: a survey by the Japan Society for Respiratory Endoscopy in 2006. *Respirology* 2009; 14: 282-9.
 7. Yang CJ, Chen TC, Hung JY, Lu PL, Sheu CC, Tsai JR, et al. Routine culture for *Mycobacterium tuberculosis* from bronchoscopy in Taiwan. *Respirology* 2007; 12: 412-5.
 8. Siddiqi K, Lambert ML, Walley J. Clinical diagnosis of smear-negative pulmonary tuberculosis in low-income countries: the current evidence. *Lancet Infect Dis* 2003; 3: 288-96.
 9. Charoenratanakul S, Dejsomritrutai W, Chaiprasert A. Diagnostic role of fiberoptic bronchoscopy in suspected smear negative pulmonary tuberculosis. *Respir Med* 1995; 89: 621-3.
 10. Jett JR, Cortese DA, Dines DE. The value of bronchoscopy in the diagnosis of mycobacterial disease. A five-year experience. *Chest* 1981; 80: 575-8.
 11. Rubins JB, Bofenkamp C. Routine culture for tuberculosis during bronchoscopy for lung cancer is not warranted. *J Bronchol* 1999; 6: 236-40.
 12. Ip M, Chau PY, So SY, Lam WK. The value of routine bronchial aspirate culture at fibreoptic bronchoscopy for the diagnosis of tuberculosis. *Tubercle* 1989; 70: 281-5.
 13. Kim MH, Suh GY, Chung MP, Kim H, Kwon OJ, Lee JH, et al. The value of routinely culturing for tuberculosis during bronchoscopies in an intermediate tuberculosis-burden country. *Yonsei Med J* 2007; 48: 969-72.

บทบาทของการส่งน้ำล้างหลอดลมเพาะเชื้อวัณโรคเป็นประจำในโรงพยาบาลมหาราชนครราชสีมา

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วัตถุประสงค์: เพื่อประเมินบทบาทของการส่งน้ำล้างหลอดลมเพาะเชื้อวัณโรคในผู้ป่วยทุกรายที่ได้รับการวินิจฉัยเป็นวัณโรคปอด และตรวจเสมหะไม่พบเชื้อตามข้อบ่งชี้ ในโรงพยาบาลมหาราชนครราชสีมา

วัสดุและวิธีการ: เป็นการศึกษาย้อนหลังแบบสังเกต โดยการเก็บข้อมูลของผู้ป่วยจำนวน 461 ราย ที่มารับการตรวจปอด และหลอดลมโดยกล้องส่องหลอดลมชนิดโค้งงอโดยคณะผู้นิพนธ์ เนื่องจากมีภาพรังสีปอดหรือเอกซเรย์คอมพิวเตอร์ปอดผิดปกติ ประกอบกับมีอาการทางระบบการหายใจ และไม่พบเชื้อวัณโรคจากการตรวจเสมหะหรือไม่มีเสมหะ ตั้งแต่วันที่ 1 ตุลาคม พ.ศ. 2551 ถึง 31 กันยายน พ.ศ. 2554 พบมีผู้ป่วยจำนวน 93 ราย ที่ได้รับการวินิจฉัยขั้นต้นเข้าได้กับวัณโรคปอด และตรวจเสมหะไม่พบเชื้อตามข้อบ่งชี้ การเพาะเชื้อวัณโรคจากน้ำล้างหลอดลมทำตามวิธีสามัญ น้ำล้างหลอดลมทุกตัวอย่างที่นำมาข้อมเชื้อไม่ได้รับการปั่นแยกน้ำและตะกอนก่อนนำมาข้อม ผู้ป่วยทุกรายในการศึกษานี้มีผลการตรวจทางพยาธิวิทยา การวิเคราะห์ทางสถิติบรรยายเป็นเปอร์เซ็นต์

ผลการศึกษา: ในจำนวนผู้ป่วย 93 ราย ที่เข้าเกณฑ์การศึกษา พบการวินิจฉัยสุดท้ายว่าเป็นวัณโรคปอดที่เสมหะไม่พบเชื้อตามข้อบ่งชี้จำนวน 87 ราย (93.5%) น้ำล้างหลอดลมที่ไม่ได้ปั่นแยกน้ำและตะกอนข้อมพบเชื้อโรค 17/93 ราย (18.3%) ขณะที่ผลเพาะเชื้อวัณโรคจากน้ำล้างหลอดลมพบเชื้อวัณโรค 34/93 ราย (36.6%), วัณโรคคือยาหลายขนานและเชื้อที่ไม่ใช่วัณโรคแต่ข้อมติดเอเอฟพีพบได้ 1/93 (1.1%) และ 5/93 ราย (5.4%) ตามลำดับ ผลการข้อมเชื้อน้ำล้างหลอดลมที่ไม่ได้ปั่นแยกตะกอนข้อมติดสี่เอเอฟพี 2/5 ราย (40%) ในผู้ป่วยที่ไม่ใช่วัณโรค (NTM) แต่ในผู้ป่วยวัณโรคคือยาหลายขนานข้อมไม่พบเอเอฟพีเลย ผลตรวจทางพยาธิวิทยาแบบแกรนูโลมาต์สพบ 18/93 ราย (19.4%) มีผู้ป่วยเพียง 5/93 ราย (5.4%) พบผลการตรวจเป็นบวกทั้งหมดในการศึกษาที่พบผู้ป่วยถึง 45/93 ราย (48.4%) ที่ตรวจไม่พบอะไรเลย

สรุป: การส่งน้ำล้างหลอดลมเพื่อเพาะเชื้อวัณโรคเป็นประจำถูกพิสูจน์ว่าควรทำในผู้ป่วยทุกรายที่ได้รับการวินิจฉัยเป็นวัณโรคปอดที่ตรวจเสมหะไม่พบเชื้อตามข้อบ่งชี้ เพราะในการศึกษานี้พบการเพาะเชื้อวัณโรคขึ้นมากที่สุด ยิ่งไปกว่านั้นการเพาะเชื้อยังสามารถใช้ในการแยกวัณโรคคือยาหลายขนาน และเชื้อที่ไม่ใช่วัณโรค (NTM) ได้ เนื่องจากไม่สามารถแยกโรคได้โดยการข้อมเอเอฟพีแต่เพียงอย่างเดียว
