



## Application of High-Frequency Oscillation during Bronchoscopy in Smear-Negative Pulmonary Tuberculosis

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### Abstract

**Background:** The diagnostic yield of bronchoalveolar lavage (BAL) for diagnosis of pulmonary tuberculosis (PTB) is low. The vibrator device is useful for sputum induction.

**Objective:** This trial was aimed to assess the value of high-frequency oscillation (HFO) during fiberoptic bronchoscopy (FOB) for diagnosis of patients with suspected PTB.

**Methods:** Suspected PTB patients with two consecutive negative sputum acid-fast bacilli (AFB) smears were recruited. Patients were chosen to use the HFO device by randomization, while the other patients underwent standard BAL. The BAL fluid and post-bronchoscopic sputum were processed for AFB smear and culture, and polymerase chain reaction for TB (PCR-TB).

**Results:** Eighty patients participating in this study, PTB was definitely diagnosed in 32 patients. The diagnostic yield of HFO with BAL culture was 27.8%, and non-HFO 21.1% ( $P = 0.71$ ). The diagnostic yield of HFO with post-bronchoscopic sputum culture was 22.2%, and non-HFO 21.1% ( $P = 1.00$ ). The diagnostic yield of PCR-TB with HFO was 33.3%, and non-HFO 21.1% ( $P = 0.47$ ).

**Conclusions:** Addition of HFO during FOB did not result in significant differences in the diagnostic yield of PTB detection in smear-negative PTB patients. However, there was a trend of increasing sensitivity of BAL PCR-TB in patients receiving HFO.

**Keywords:** high-frequency oscillation, bronchoscopy, smear-negative pulmonary tuberculosis

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## Introduction

Pulmonary tuberculosis (PTB) remains an important cause of health problems in the world. WHO has recommended testing for acid-fast bacilli (AFB) in sputum specimens for the diagnosis of pulmonary tuberculosis<sup>1</sup>. Approximately 50% of pulmonary tuberculosis cases have a negative sputum smear for AFB<sup>2</sup>. However, there are many patients with suspected pulmonary tuberculosis but can expectorate by themselves. In regions of high PTB prevalence, when the clinical diagnosis of PTB is likely, empirical treatment is the best course of action, while bronchoalveolar lavage (BAL) is reserved for further investigation of nonresponders. Nevertheless, the sensitivity of BAL for a definitive diagnosis of PTB (i.e. a positive culture result)<sup>3</sup> has remained low, ranging from 15 - 40%<sup>4,5</sup>.

In recent years, respiratory therapy devices have been offered as a standard choice of treatment. High-frequency vibration devices help expectorate phlegm out of the respiratory tract. The rapid vibration placed over the chest wall can also reduce the viscosity of the mucus<sup>6-8</sup>. It is hypothesized that the mechanism of high-frequency vibration that enhances expectoration is by increasing the surface area of the airway and alveolar walls with secretions, and by reducing the viscosity of sputum.

The purpose of this study was to evaluate the diagnostic yield of high-frequency oscillation (HFO) for BAL sampling in the detection of tuberculosis, in comparison with non-HFO, in terms of standard BAL culture for *M. tuberculosis* (TB), BAL polymerase chain reaction for TB (PCR-TB), and post-bronchoscopic sputum AFB smear and culture for TB in patients suspected of PTB but with a negative sputum smear.

## Materials and Methods

This randomized controlled trial was conducted in adult patients who underwent diagnostic fiberoptic bronchoscopy (FOB) for PTB during November 2013 to November 2014 at the Division of Pulmonary and Critical Care, Ramathibodi Hospital, Bangkok. The study was reviewed and approved by the Committee on Human Rights Related to Research Involving Human Subjects, Ramathibodi Hospital, Mahidol University. Informed consent was obtained from patients.

### Subjects

Patients were 15 years of age or older. All patients who were suspected of PTB based on clinical findings and chest radiography (CXR) and with two consecutive negative sputum AFB smears were included in the study. We excluded cases with contraindication for bronchoscopy and/or use of high-frequency vibration. Exclusion criteria included: head and/or neck injury which had not been stabilized, active hemorrhage with hemodynamic instability, temporary pacemaker, acute pulmonary embolism, hemoptysis, emphysema, untreated pneumothorax, and fractured ribs in the area needing to be percussed.

### Study design

All patients underwent bronchoscopic examinations with BAL and/or transbronchial biopsy, performed at the indicated segments. BAL technique was performed according to technical recommendations and guidelines for BAL procedure from the American Thoracic Society (2004) and the European Respiratory Society (2011). Patients were selected to use the HFO device by randomization (using blocks of four stratified by radiologic pattern, i.e. patchy, reticular, nodular, and reticulonodular infiltrate), while a control group

underwent standard BAL. A chest percussion device (Bunn Medavibe®; General Physiotherapy, USA), with an ultra-light applicator head and a continuously variable speed range of 20–30 cycles per second, was used during the procedure. During the FOB procedure, the device was placed over the chest wall, with the bronchoscope in wedge position within each desired segment for at least 30 seconds before the specimen was obtained. The BAL specimens were processed for cell count/differentiation, AFB smear and culture, and PCR-TB. One post-bronchoscopic sputum sample was also sent for AFB smear and culture. The diagnostic yield of each group was calculated by comparing positive results from any of the detection methods described above with the definite diagnosis of PTB (see below).

### Definite diagnosis of PTB

1. Positive BAL culture for TB
2. Granuloma found in the pathology specimen obtained by transbronchial biopsy
3. Positive post-bronchoscopic sputum culture for TB
4. Smear-negative and culture-negative patients who responded to anti-TB drugs, defined by improvement of clinical symptoms and CXR

### Statistical analysis

Statistical analyses were performed using Stata software (StataCorp. 2013. Stata Statistical Software. Release 13. College Station, TX: StataCorp LP.) Categorical data were reported as percentage and continuous data were reported as mean  $\pm$  SD or median and interquartile range. Comparisons of baseline characteristics between the two groups of PTB patients were performed using chi-square test or Fisher's exact

test for categorical data and Student's *t*-test or Mann-Whitney *U* test for continuous data. A *P* - value less than 0.05 was considered to be statistically significant.

## Results

Of the 80 patients participating in the study, PTB was definitely diagnosed in 32 patients. Baseline characteristics, symptoms, chest X-ray pattern and definite diagnosis of patients are described in Table 1.

The most common clinical feature in the study participants was cough (40%), followed by asymptomatic, dyspnea, hemoptysis, weight loss, and fever. Dyspnea was higher in the HFO group than in the non-HFO group (7 vs. 1 patients, respectively, *P*=0.02), while hemoptysis was higher in the non-HFO group than in the HFO group (6 vs. 1 patients, respectively, *P* = 0.04); otherwise, there were no significant differences in symptoms between the HFO and non-HFO groups.

In the definite diagnosis, the most common X-ray lesion was reticulonodular infiltration, which was found in 17 (21%) patients (8 cases in the HFO group, and 9 cases in the non-HFO group), followed by 9 (11%) patients with patchy infiltration (4 HFO, 5 non-HFO), 7 (9%) with nodular (5 HFO, 2 non-HFO), and 3 (4%) with reticular (1 HFO, 3 non-HFO).

In our study where PTB was definitely diagnosed in 32 (40%) patients, we found positive BAL culture for TB in 9 (11.25%) cases (5 patients in the HFO group, and 4 in the non-HFO group). In 4 (5%) patients (3 HFO, 1 non-HFO) granuloma was found in the pathology specimen obtained by transbronchial biopsy. Positive post-bronchoscopy sputum (PBS) culture for TB was found in 8 (10%) patients (4 HFO, 4 non-HFO). Smear-negative and culture-negative patients who responded to anti-TB drugs (defined by improvement of clinical symptoms and CXR) totaled 20 (25%) patients

(10 HFO, 10 non-HFO). Additionally, we found positive BAL culture for nontuberculous mycobacteria (NTM) in 7 (8.75%) patients (3 HFO, 4 non-HFO), and positive PBS culture for NTM in 4 (5%) patients (2 HFO, 2 non-HFO). Results of specimen examination in each group are shown in Table 2.

The diagnostic yield of HFO and non-HFO during FOB in sputum smear-negative TB suspects is summarized in Table 3. The addition of HFO during FOB did not increase the overall diagnostic yield

( $P = 0.47$ ) or the diagnostic yield when analyzed by subgroup, i.e. BAL culture ( $P = 0.71$ ), PBS culture ( $P = 1.00$ ), and BAL PCR-TB ( $P = 0.47$ ). However in this study, no complications occurred among patients undergoing bronchoscopy.

The diagnostic yield of HFO and non-HFO during FOB analyzed by subgroup and stratified by radiologic pattern is summarized in Figure 1. Comparisons of the diagnostic yield by subgroup showed no significant differences.

**Table 1** Baseline characteristics of subjects with suspected pulmonary tuberculosis

Characteristics		HFO (N = 40)	Non-HFO (N = 40)	P - value
<b>Male sex - n (%)</b>		21 (53)	22 (55)	0.82
<b>Mean age - yr (<math>\pm</math> SD)</b>		56 ( $\pm$ 2.31)	60 ( $\pm$ 2.21)	0.22
<b>Underlying disease - n (%)</b>	<b>None</b>	16 (40)	14 (35)	0.64
	<b>DM</b>	4 (10)	3 (8)	0.69
	<b>HT</b>	7 (18)	8 (20)	0.77
	<b>CRF</b>	1 (3)	0	0.31
	<b>CLD</b>	5 (13)	7 (18)	0.39
	<b>Other</b>	7 (18)	8 (20)	0.82
<b>Contact TB - n (%)</b>		3 (8)	1 (3)	0.30
<b>Former smoker - n (%)</b>		8 (20)	5 (13)	0.39
<b>History of PTB - n (%)</b>		5 (13)	9 (23)	0.39
<b>Symptom - n (%)</b>	<b>Asymptomatic</b>	17 (43)	12 (30)	0.24
	<b>Dyspnea*</b>	7 (18)	1 (3)	0.02
	<b>Cough</b>	12 (30)	20 (50)	0.06
	<b>Fever</b>	1 (3)	0	0.31
	<b>Hemoptysis*</b>	1 (3)	6 (15)	0.04
	<b>Weight loss</b>	2 (5)	1 (3)	0.55
<b>Infiltration pattern - n (%)</b>	<b>Patchy</b>	7 (18)	10 (25)	0.81
	<b>Reticular</b>	5 (13)	8 (20)	0.39
	<b>Nodular</b>	9 (23)	4 (10)	0.36
	<b>Reticulonodular</b>	19 (48)	18 (45)	0.91
<b>Lesion severity score**</b>				
<b>median (range)</b>		2.5 (0-12)	2.6 (1-7)	0.87
<b>Treatment - n (%)</b>		19 (47.50)	21 (52.50)	0.65
<b>Definite diagnosis - n (%)</b>		18 (45.0)	19 (47.5)	0.82

DM, diabetes mellitus; HT, hypertension; CRF, chronic renal failure; CLD, chronic lung disease.

\* Significant difference between groups.

\*\* Lesion severity scoring from X-ray images<sup>9</sup>.

**Table 2** Results of specimen examination in each group

Result	HFO (N = 40)	Non-HFO (N = 40)	P - value
<b>AFB BAL positive - n (%)</b>	1 (2.5)	0 (0)	1.00
<b>AFB post-BAL positive - n (%)</b>	1 (2.5)	2 (5.0)	1.00
<b>C/S BAL for TB positive - n (%)</b>	5 (12.5)	4 (10.0)	1.00
<b>C/S post-BAL for TB positive - n (%)</b>	4 (10.0)	4 (10.0)	1.00
<b>BAL PCR-TB positive - n (%)</b>	6 (15.0)	4 (10.0)	0.85
C/S BAL for NTM positive - n (%)	3 (7.5)	4 (10.0)	0.90
C/S post-BAL for NTM positive - n (%)	2 (5.0)	2 (5.0)	1.00
<b>Volume (ml) -</b>	<b>In</b>	114.25 ( $\pm$ 20.24)	109.75 ( $\pm$ 28.42)
<b>mean (<math>\pm</math> SD)</b>	<b>Out</b>	22 ( $\pm$ 9.53)	28.5 ( $\pm$ 12.26)
<b>Duration of FOB (sec) -</b>		30 (10-60)	30 (10-60)
<b>median (range)</b>			0.89
<b>Cell count - median (IQR)</b>		518 (255-1,180)	294 (85-1,004)
<b>Granuloma - n (%)</b>		3 (7.5)	1 (2.5)
			0.90

AFB, acid-fast bacilli; BAL, bronchoalveolar lavage; C/S, culture; PCR-TB, polymerase chain reaction for TB; NTM, non-tuberculous mycobacterium.

**Table 3** Diagnostic yield of the two different diagnostic modalities

Variables	HFO (N = 40)	Non-HFO (N = 40)	P - value
<b>Overall - n (%)</b>	33.3	21.1	0.47
<b>BAL culture - n (%)</b>	27.8	21.1	0.71
<b>PBS culture - n (%)</b>	22.2	21.1	1.00
<b>BAL PCR-TB - n (%)</b>	33.3	21.1	0.47

BAL, bronchoalveolar lavage; PBS, post-bronchoscopy sputum; PCR-TB, polymerase chain reaction for TB.

## Discussion

Fiberoptic bronchoscopy is commonly performed in patients suspected of pulmonary TB who either cannot expectorate or who have had negative sputum smear results<sup>10</sup>. A major advantage of bronchoscopy in suspected patients with negative sputum smear examination for AFB is the isolation of mycobacteria at an early stage when the destruction of lung tissue is minimal and the risk of spreading the disease to others can be decreased by early diagnosis and treatment.

The effect of volume instilled and the percentage of the returned amount have been shown to be important. Aspiration of less than 5% of the volume instilled is considered to be an inadequate alveolar sample<sup>11</sup>. In one study of bronchoscopic lavage, it was shown that there was a higher diagnostic yield in patients with a greater than 5% return of instilled fluid<sup>12</sup>. Our study showed mean fluid returned of 22 ml in HFO and 28.5 ml in non-HFO, which was considered to be adequate in both groups (more than 5%). The fluid return in the HFO

group was significantly less than in the non-HFO group. This may be explained by the effect of HFO technique, which could cause the bronchoscopic tip to move and partially dislodge from the wedge position, causing fluid leakage into the proximal airway.

In a study comparing differential cell counts, De Brauwer et al. determined that the presence of 300 to 500 cells per ml of fluid provided a good representation for a BAL sample<sup>13</sup>. In our study the median cell counts were 518 (IQR 255 - 1,180) cells per ml in HFO and 294 (IQR 85 - 1,004) cells per ml in non-HFO ( $P = 0.10$ ). We concluded that the specimens were acceptable, and thus the results of our study should not be compromised by the inadequacy of the specimens.

Regarding the diagnostic yield of BAL in the diagnosis of PTB, Wallace et al., Kennedy et al. and Vijayan et al.<sup>4, 14, 15</sup> demonstrated lower yield in their studies as compared with ours, whereas Baughman et al.<sup>16</sup> reported 87% sample positivity for bronchoscopy in sputum smear-negative cases. In Thailand, Charoenatankul et al. performed BAL in 40 patients suspected to have pulmonary tuberculosis, whose chest roentgenograms revealed minimal infiltration and with sputum smears negative for AFB, and found the diagnostic yield of overall bronchoscopic procedures (BAL culture) to be 15%<sup>17</sup>. In this study, we demonstrated that the result of HFO and non-HFO during FOB for the diagnosis of patients with suspected PTB had diagnostic yield of 27.8% and 21.1%, respectively. Our study showed no difference in diagnostic yield for diagnosis of PTB compared with previous studies. Also, the use of HFO during FOB did not increase the diagnostic yield ( $P = 0.71$ ).

In Iran, Malekmohammad et al. demonstrated that additional PBS study obtained after the bronchoscopy can variably increase the sensitivity of BAL microscopy,

with diagnostic yield for positive BAL culture of *Mycobacterium tuberculosis* from 57.1 to 83.9%<sup>18</sup>. In this study, we demonstrated that the result of HFO and non-HFO during FOB for the diagnosis of patients with suspected PTB had the diagnostic yield of 22.2% and 21.1%, respectively. And the addition of HFO study during FOB did not increase the diagnostic yield ( $P = 1.00$ ). Our study showed lower diagnostic yield than for diagnosis PTB with previous studies. There may be some explanation for the low sensitivity of BAL C/S for TB. Sputum smear-negative pulmonary tuberculosis is a paucibacillary condition, and the dilution of epithelial lining fluid by the instilled saline might be responsible for the low yield from BAL specimens. In addition, the local anesthetic used for bronchoscopy might also suppress the growth of *M. tuberculosis*<sup>19</sup>.

In our study, the majority of participants had undergone chest computed tomography (chest CT) before FOB: 67 (83.8%) patients (33 cases in HFO group and 34 cases in non-HFO group). The main findings on the chest CT in both groups were a tree-in-bud pattern. The areas of abnormality were minimal, which may affect the diagnostic yield. We found abnormalities on the chest CT and definite diagnosis of PTB in 31 (38.8%) patients (14 cases in the HFO group and 17 cases in the non-HFO group).

In regard to the clinical utility of rapid tuberculosis detection in BAL samples by PCR, it was observed that BAL PCR with HFO gave a diagnostic yield of 33.3%, while non-HFO gave 21.1% ( $P = 0.47$ ). This indicates that BAL PCR has good accordance with increased diagnosis of active tuberculosis. In a previous study, PCR assay gave a positivity rate of 80.9% compared with 8.8% for smear examination and 7.4% for culture for *M. tuberculosis* in BAL specimens<sup>20</sup>. Thus, PCR



assay was found to be more sensitive than smear and culture for the detection of *M. tuberculosis* in BAL specimens of patients with sputum smear-negative PTB.

An advantage of this study was that it was a randomized controlled trial, and there had never been a study of this kind before. Additionally, there was a chance of increasing the diagnostic yield of BAL PCR for TB in the patients receiving HFO vs. non-HFO, although as it turned out there was no significant difference between the two groups.

Limitations of the HFO in our study may be caused by two reasons. First, the FOB technique showed splash-out of fluid during wedging in HFO that resulted in less fluid return; also, the wedging duration may vary. Secondly, it was difficult to use HFO during FOB

in the posterior aspect of the chest wall, where post-primary TB within the lungs had developed in majority of cases (i.e. either in posterior segments of the upper lobes or superior segments of the lower lobes<sup>21, 22</sup>). In a future study, we may increase the HFO frequency during FOB, or change to the use of a high-frequency chest wall oscillation vest.

## Conclusions

The addition of HFO during FOB did not result in a significant difference in the sensitivity of PTB detection in smear-negative PTB patients. However, there was a trend of increasing diagnostic yield of BAL PCR for TB in the patients receiving HFO. Further studies with a larger study population may be needed.

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## Original Articles/นิพนธ์ต้นฉบับ

# การใช้อุปกรณ์การสั่นความถี่สูงระหว่างการส่องกล้องหลอดลมในผู้ป่วยสงสัยวัณโรคปอดที่เสนอหัตถการย้อมสีทันกรดเป็นลบ

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## บทคัดย่อ

**ความเป็นมา:** Diagnostic yield ของการทำหัตถการส่องกล้องหลอดลม (Bronchoalveolar lavage; BAL) ในการวินิจฉัยวัณโรคปอดค่อนข้างต่ำ โดยอุปกรณ์การสั่นความถี่สูงมีประโยชน์ในการกระตุ้นเพื่อขับเสมหะ

**วัตถุประสงค์:** เพื่อประเมินคุณค่าของการตรวจวินิจฉัยวัณโรคจากการใช้อุปกรณ์การสั่นความถี่สูง (High-frequency oscillation; HFO) ระหว่างการส่องกล้องหลอดลม เพื่อช่วยวินิจฉัยในผู้ป่วยที่สงสัยวัณโรคปอด

**วิธีการศึกษา:** ผู้ป่วยที่แพทเทิลส์สงสัยว่าเป็นวัณโรคปอดและมีผลการเก็บเสมหะตรวจย้อมสีทันกรด (AFB) เป็นลบ อย่างน้อยสองครั้ง ซึ่งผู้ป่วยที่ได้รับการคัดเลือกจะมีการใช้ HFO แบบสุ่ม ขณะที่ผู้ป่วยอีกกลุ่มจะไม่ได้มีการใช้ HFO ระหว่าง BAL โดยน้ำสีล้างปอดของผู้ป่วยทั้งสองกลุ่มจะส่งตรวจย้อมสีทันกรด เพาะเชื้อวัณโรคและ PCR สำหรับวัณโรค

**ผลการศึกษา:** ผู้ป่วยแปดสิบรายเข้าการศึกษานี้ โดยหลังสิ้นสุดการศึกษามีผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็นวัณโรคปอด 32 ราย พบว่า diagnostic yield ของการเพาะเชื้อวัณโรคของกลุ่ม HFO 27.8%, และ non-HFO 21.1% ( $P = 0.71$ ) ส่วน diagnostic yield ของการเพาะเชื้อวัณโรคจากเสมหะหลังการส่องกล้องหลอดลมของกลุ่ม HFO 22.2%, และ non-HFO 21.1% ( $P = 1.00$ ) และในส่วน diagnostic yield ของการตรวจ PCR-TB ของกลุ่ม HFO 33.3%, และ non-HFO 21.1% ( $P = 0.47$ )

**สรุป:** การเพิ่มอุปกรณ์ HFO ระหว่างการส่องกล้องหลอดลมพบว่า ไม่มีความแตกต่างกันของจำนวนปั๊มสำคัญในการช่วยวินิจฉัยวัณโรคปอดในผู้ป่วยที่เสนอหัตถการย้อมสีทันกรดเป็นลบ อย่างไรก็ตาม มีแนวโน้มที่จะมีความไวเพิ่มขึ้นในกลุ่มที่ใช้ HFO ร่วมกับการตรวจ PCR-TB

**คำสำคัญ:** อุปกรณ์การสั่นความถี่สูง หัตถการส่องกล้องหลอดลม วัณโรคปอดที่เสนอหัตถการย้อมสีทันกรดเป็นลบ

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