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Original article

The role of smear microscopy of induced sputum and bronchoalveolar lavage in the diagnosis of pulmonary tuberculosis in patients with initial smear-negative: A prospective study

Ngoc Minh Duong[a], Khoa Dang Nguyen[a], Nguyen Ngoc Tran[b], Vu Thuong Le[a*]

[a]*Department of Internal Medicine, University of Medicine and Pharmacy at Ho Chi Minh City; 217 Hong Bang Street, Ward 11, District 5, Ho Chi Minh City, Vietnam;*
[b]*Department of Tuberculosis and Pulmonary Disease, University of Medicine and Pharmacy at Ho Chi Minh City; 217 Hong Bang Street, Ward 11, District 5, Ho Chi Minh City, Vietnam*.

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Abstract: ***Introduction:*** Several studies have compared the diagnostic value of sputum induction (SI) with flexible fiberoptic bronchoscopy (FOB) in diagnosing pulmonary tuberculosis; however, these investigations yield an inconsistent conclusion. This study aims to evaluate the role of acid-fast bacilli (AFB) testing of SI and bronchoalveolar lavage (BAL) samples in suspected pulmonary tuberculosis cases. ***Methods:*** A prospective study was conducted at the Department of Pulmonary in Cho Ray Hospital (Ho Chi Minh City, Vietnam) between October 2020 and May 2021. The study comprised 60 patients hospitalized with suspected pulmonary tuberculosis who had at least one negative AFB result from spontaneous sputum or gastric lavage. All participants underwent AFB testing of SI and BAL samples on the same day. ***Results:*** Among 60 patients, 25 (41.7%) were diagnosed with pulmonary tuberculosis. Of the patients with pulmonary tuberculosis, 13 had positive AFB results, including four cases with both positive AFB SI and positive AFB BAL results. The sensitivity of AFB SI was significantly lower compared to that of AFB BAL (16% vs. 52%, p = 0.0027). The most common complication associated with the SI method was cough (15%). The proportion of patients able to provide sputum using the SI method was significantly higher than those with spontaneous sputum (p = 0.0499, McNemar test). ***Conclusions:*** SI is a safe and effective method for collecting respiratory specimens, even from patients unable to expectorate spontaneous sputum. FOB should be reserved for suspected cases of pulmonary tuberculosis that are negative for AFB in spontaneous sputum, SI, and gastric lavage.

Keywords: tuberculosis, sputum induction, nebulizer, acid-fast bacilli, bronchoalveolar lavage.

1. INTRODUCTION

Despite the availability of vaccinations for over 90 years and more than 60 therapeutic years, tuberculosis remains the most common cause of infection-related death, surpassing human immunodeficiency virus [1]. Vietnam is among the 30 nations with the highest tuberculosis burden worldwide and has a high disease prevalence, estimated at 7.4% [2]. Sputum smear microscopy is still the primary method for diagnosing tuberculosis in Vietnam and other low- and middle-income countries. Unfortunately, only approximately 50% to 60% of patients with active tuberculosis are detected by this approach [3]. Microscopic screening of sputum samples for AFB has been used extensively worldwide to diagnose tuberculosis. Patients with negative sputum AFB results but positive

*Address correspondence to Vu Thuong Le at Department of Internal Medicine, University of Medicine and Pharmacy at Ho Chi Minh City; 217 Hong Bang Street, Ward 11, District 5, Ho Chi Minh City, Vietnam; E-mail: lethuongvu1971@gmail.com

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respiratory specimen cultures can transmit the disease. Therefore, individuals in contact with patients with negative sputum AFB results are still at risk of being contaminated with tuberculosis and developing active illness [4].

SI has long been acknowledged as a reliable method for diagnosing pulmonary tuberculosis, with tolerability superior to gastric lavage testing in patients who are unable to expectorate sputum. Until now, SI has been primarily neglected with the development of FOB [5]. Unfortunately, FOB is an invasive and expensive diagnostic technique that is unavailable in many health facilities, particularly at the provincial hospital. Several studies have attempted to compare the diagnostic value of SI with FOB in diagnosing pulmonary tuberculosis; however, these investigations yield an inconsistent conclusion [5-9]. Furthermore, SI has several advantages over FOB, such as repeatability, better patient tolerance, fewer complications, and greater feasibility. SI can be performed in medical facilities with limited equipment and human resources, particularly in developing countries [6, 7, 10]. This study aims to evaluate the diagnostic value of AFB testing of SI and BAL samples by FOB in suspected pulmonary tuberculosis cases with initial AFB negative results from spontaneous sputum or gastric lavage. The study also compared the complications of SI process to the FOB procedure and examined the rate of samples of appropriate quality obtained using SI compared to spontaneous sputum.

2. METHOD

A prospective, single-center study was conducted at the Department of Pulmonary in Cho Ray Hospital in Ho Chi Minh City, Vietnam, between October 2020 and May 2021. The study comprised 60 consecutive patients admitted to the hospital with suspected pulmonary tuberculosis who had at least one negative AFB result from spontaneous sputum or gastric lavage at the outpatient clinic. These patients were identified based on the World Health Organization's definition of suspected pulmonary tuberculosis cases [11, 12]. All participants in this study underwent AFB testing of specimens from SI and BAL on the same day.

2.1. Inclusion criteria

The study enrolled patients who met all the following criteria: i) aged 18 years and older and consented to participate in the study, ii) were suspected of suffering active pulmonary tuberculosis based on symptoms including productive cough, dyspnea, chest pain, hemoptysis, or constitutional symptoms such as loss of appetite, weight loss, fever, night sweats, fatigue, and symptoms that persisted for more than two weeks [11, 12], iii) had abnormal imaging on chest X-ray, iv) had at least one negative AFB result from spontaneous sputum or gastric lavage.

2.2. Exclusion criteria

The study had exclusion criteria, which included: i) patients with contraindications to SI collection, such as a history of significant epistaxis requiring hospitalization, coagulopathy, heart failure, pneumothorax, recent ocular surgery, or severe asthma requiring admission to intensive care unit [13], ii) patients with contraindications to FOB, including severe hypoxia, acute asthma, exacerbation of chronic obstructive pulmonary disease, current or recent myocardial infarction, uncontrolled congestive heart failure, life-threatening cardiac arrhythmias, and severe coagulopathy [14].

2.3. Sputum induction

SI aims to receive samples of lower respiratory tract secretions from patients who cannot expectorate sputum without performing FOB. Hypertonic saline generates aerosolized particles that settle in the airways and induce interstitial fluid displacement into the airways due to osmotic pressure [15]. These secretions within the airways can be coughed up and examined for AFB [9]. SI collection is a safe procedure with a reasonable risk of bronchospasm [16].

For patients with a history of asthma or chronic obstructive pulmonary disease, salbutamol 200-400µg spray is administered prior to SI collection. The procedure involved nebulizing a 5ml of hypertonic saline 3% solution with a jet nebulizer. The procedure is repeated if sputum cannot be obtained after the first nebulization. Patients are encouraged to cough and expectorate sputum every 5 minutes during nebulization until a minimum of 2ml of sputum is produced [17]. The procedure is considered failure if, after 15-20 minutes of the later nebulization, the patient cannot cough up sputum or develops complications such as cough, chest tightness, wheezing, or dyspnea due to bronchospasm, necessitating discontinuation of the procedure. Within 30 minutes, the collected sputum samples are transmitted to the Department of Microbiology, where they are assessed for quality and subjected to AFB fluorescence staining. The AFB results will be read by two experienced technicians, who were blinded to all patient information, including the final diagnosis.

2.4. Flexible fiberoptic bronchoscopy

The patient experienced FOB using an Olympus CV-170 bronchoscope (Japan) and was injected subcutaneously with atropine 0.25mg and nebulized with 2% lidocaine 2ml and fenoterol/ipratropium 1ml prior to the procedure. Guidance for the location of BAL was determined by utilizing X-ray or computed tomography of the chest when available. The procedure involves performing BAL with normal saline at room temperature, with a volume ranging from 100 to 300ml. If abnormalities of the bronchial mucosa are observed during FOB, a biopsy of the affected tissue is performed to aid in the definitive diagnosis. Following BAL, the collected fluid is promptly transported to the Department of Microbiology and tested, including AFB fluorescence staining and mycobacteria growth indicator tube (MGIT) culture.

2.5. Case and variable definitions

Pulmonary tuberculosis was defined based on positive AFB (including SI or BAL samples), positive MGIT BAL culture for *Mycobacteria tuberculosis*, or tuberculosis tissue identified in bronchial mucosal specimens by histopathology. AFB results are considered positive when there are more than 1 *Mycobacterium* bacteria per 100 fields.

Complications during SI collection were defined as follows: i) severe coughing to suspend the procedure, ii) bronchospasm characterized by severe dyspnea, wheezes, and requiring bronchodilator administration and discontinuation

of the procedure, and iii) vomiting accompanied by nausea and inability to continue the procedure.

Complications during FOB are defined as follows: i) hypoxemia: a decrease of SpO_2 > 5% compared to the pre-procedure level or SpO_2 < 90% [14], ii) bleeding: requiring hemostasis with adrenaline, iii) bronchospasm: severe dyspnea, wheezes, requiring bronchodilators and termination of the procedure, iv) fever: temperature > 38°C within 24 hours after the procedure, v) infection progression: worsening infection within 24-48 hours after FOB, requiring a modification in antibiotic therapy, vi) pneumothorax: confirmed by chest X-ray or computed tomography.

2.6. Study protocol

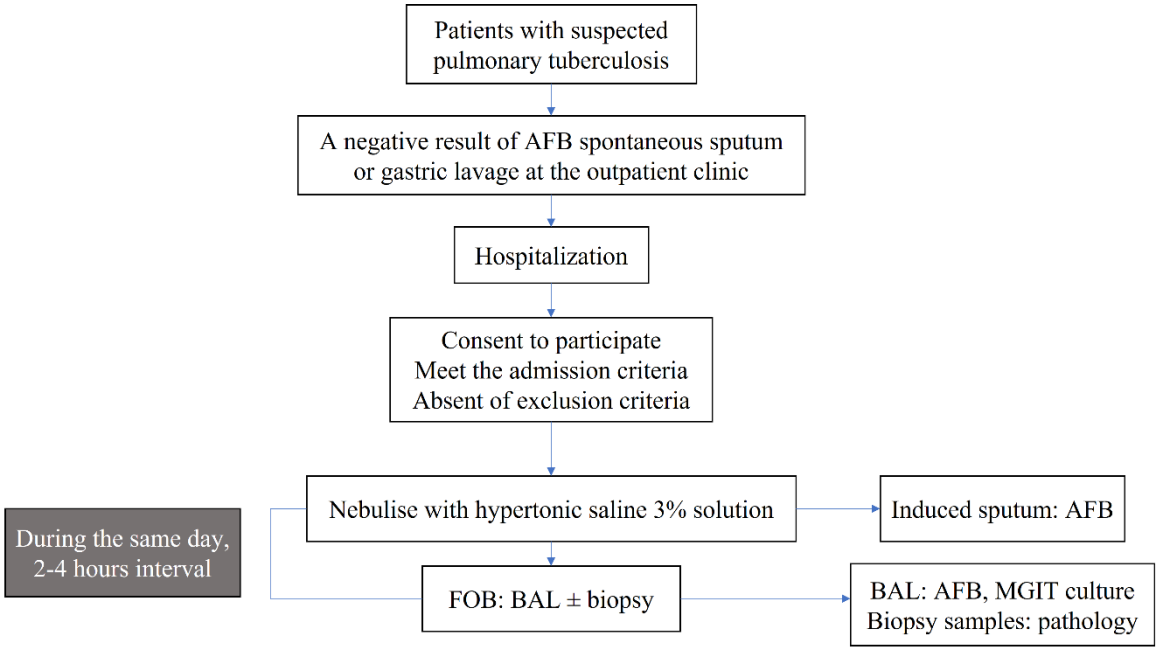

Figure 1. Study protocol

2.7. Statistical analysis

The statistical analysis was performed using Stata version 15.1 (StataCorp). Qualitative variables are reported as frequencies and percentages, while quantitative variables are presented as mean ± standard deviation (SD) (if normally distributed). The relationship between qualitative variables was evaluated by Chi-squared test or Fisher's exact test, while the t-test was used to evaluate the association between normally distributed quantitative variables. Paired data proportions within the same population were compared using the McNemar test. Statistical significance was set at $p < 0.05$.

3. RESULTS

3.1. Characteristics and etiology of the study populations

The study included 60 patients, of whom 25 (41.7%) were diagnosed with pulmonary tuberculosis, and 35 (58.3%) were diagnosed with other etiologies. Our study did not have any cases with an indeterminate index test result, which is defined as having 1 to 2 acid-fast bacilli in the entire smear sample. Pneumonia was the most common among patients diagnosed with etiologies other than pulmonary tuberculosis, accounting for 82.9% of cases. Both groups had a male predominance, with percentages ranging from 60% to 68.6%. No statistically significant difference was observed between the two groups regarding age or symptoms such as fever, non-productive cough, dyspnea, productive cough, hemoptysis, and chest pain. In the pulmonary tuberculosis group, fever was recorded as the most frequently occurring symptom, accounting for 72%, while in the group with other diagnoses, the incidence of fever exceeded 50%. Among the patients with pulmonary tuberculosis, 13 had positive AFB, with four cases having simultaneously positive AFB results from SI and BAL. The remaining 12 cases of pulmonary tuberculosis were diagnosed through histopathology or MGIT culture of BAL positive for *Mycobacteria tuberculosis*. A detailed report of the study population's baseline, clinical, and laboratory characteristics can be found in table 1.

3.2. Complications of FOB procedure and SI collection

Regarding the SI method, the most common complication was cough, which occurred in 15% of cases, while 2 out of 60 cases (3.3%) experienced bronchospasm. Furthermore, hypoxemia was the most common complication of FOB, which accounted for 26.7% of cases. Other complications, such as fever, bronchospasm, and hemorrhage, were also recorded at 8.3%, 6.6%, and 5%, respectively (as shown in table 2).

Table 2. Complications of flexible fiberoptic bronchoscopy and sputum induction collection of the study population (n = 60)

Complication	Sputum induction (n, %)	Flexible fiberoptic bronchoscopy (n, %)
Cough	9/60 (15%)	
Vomit	0/60 (0%)	
Bronchospasm	2/60 (3.3%)	4/60 (6.6%)
Hypoxemia		16/60 (26.7%)
Hemorrhage		3/60 (5%)

Fever	5/60 (8.3%)
Infection progression	0/60 (0%)
Pneumothorax	0/60 (0%)

3.3. Comparing the effectiveness of sputum collection between SI and spontaneous sputum method

Among the 60 cases participating in the study, 36 cases (60%) were able to collect spontaneous sputum samples at the outpatient clinic. In comparison, 46 cases (76.7%) collected sputum samples through SI method. Six patients were unable to collect sputum using either of the above approaches. The proportion of patients who collected sputum using the SI method was statistically significantly higher than spontaneous sputum method ($p = 0.0499 < 0.05$, McNemar test), as shown in table 3.

Table 3. Comparing the effectiveness of sputum collection between SI and spontaneous sputum method (n = 60)

Methods	Induced sputum (+)	Induced sputum (-)	p
Spontaneous sputum (+)	28	8	0.0499*
Spontaneous sputum (-)	18	6	

**McNemar test*

Table 1. Baseline, clinical, and laboratory characteristics of the study population (n = 60)

Variables	Pulmonary TB (n = 25)	Non-pulmonary TB (n = 35)	p
Sex			
Male (n, %)	15/25 (60%)	24/35 (68.6%)	
Female (n, %)	10/25 (40%)	11/35 (31.4%)	
Age (mean ± SD)	57.4 ± 17.5	58 ± 17	0.782[a]
Symptom			
Fever (n, %)	18/25 (72%)	18/35 (51.4%)	0.181[b]
Non-productive cough (n, %)	12/25 (48%)	12/35 (34.3%)	0.301[b]
Dyspnea (n, %)	8/25 (32%)	15/35 (42.9%)	0.432[b]
Productive cough (n, %)	8/25 (32%)	13/35 (37.1%)	0.786[b]
Hemoptysis (n, %)	3/25 (12%)	6/35 (17.1%)	0.722[c]
Chest pain (n, %)	2/25 (8%)	6/35 (17.1%)	0.449[c]
Definitive diagnosis			
Pulmonary TB AFB (+) (n, %)	13/25 (52%)		
Induced sputum (n, %)	4/25 (16%)		< 0.05[c]
BAL (n, %)	13/25 (52%)		
Pulmonary TB AFB (-)[d] (n, %)	12/25 (48%)		
Pneumonia (n, %)		29/35 (82.9%)	
Bronchiectasis (n, %)		2/35 (5.7%)	
Lung cancer (n, %)		2/35 (5.7%)	
CTD-ILD (n, %)		1/35 (2.9%)	
Nontuberculous mycobacteria (n, %)		1/35 (2.9%)	

[a]T-test, [b]Chi-square test, [c]Fisher's exact test, [d]Histopathology confirmed pulmonary tuberculosis or MGIT culture of BAL was positive for *Mycobacteria tuberculosis*

4. DISCUSSION

The main findings of our study revealed that in hospitalized patients with suspected pulmonary tuberculosis who had at least one negative AFB result from spontaneous sputum or gastric lavage, the sensitivity of AFB SI was significantly lower compared to that of BAL (16% vs. 52%, p = 0.0027). Additionally, the SI method had fewer significant complications than FOB, and the rate of successfully obtaining lower respiratory tract secretions was significantly higher than spontaneous sputum collection.

The study conducted by Loh revealed that only one patient out of 16 had forced expiratory volume in one second (FEV_1) reduction of more than 20% after nebulized 4% hypertonic saline solution [18]. Covar also demonstrated the safety of nebulizing hypertonic saline solution for SI collection in pediatric patients with asthma and concluded that this is a non-invasive and safe technique, with bronchospasm occurring in only 9 out of 117 cases (7.7%) [19]. The mechanism behind bronchospasm induced by inhaling particles of hypertonic saline solution is undefined but possibly related to sensory nerve endings. Furthermore, particles of distilled water have also been reported to cause bronchospasm [20]. Nevertheless, the incidence of bronchospasm observed in our study was relatively low, indicating that the SI method is safe and acceptable.

Our study found that 76.7% of patients could produce sputum samples after nebulizing hypertonic saline solution, and 75% of patients who were unable to expectorate spontaneous sputum could have sputum after using the SI method. Loh's research showed a 100% success rate in receiving sputum samples using the SI approach; however, the study was performed on 16 healthy individuals with lower acceptability [18]. In a systematic review of 23 studies, the rates of obtaining sputum samples after nebulized hypertonic saline solution ranged from 76% to 100% [21].

The success rate of obtaining samples via SI depends on the type of nebulizer, concentration, and volume of hypertonic saline solution. Previous studies have demonstrated higher success rates using ultrasonic nebulizers than jet nebulizers [22]. Our study utilized a jet nebulizer; therefore, the success rate of sputum collection may be lower. Additionally, we used a 3% hypertonic saline solution with a volume of 5-10ml. International variation in the volume and concentration of the hypertonic saline solution used for SI exists. The concentration of hypertonic saline solution ranges from 3%-7%, while the volume ranges from 5-90ml in several studies [6, 8, 23, 24]. Therefore, comparing different SI strategies and establishing a standardized protocol worldwide is necessary.

Park's study on 39 patients with pulmonary tuberculosis showed that only seven patients (17.9%) had positive AFB using SI [25]. Rao reported that AFB testing with SI was positive in 76/120 (63.3%) patients [26]. Furthermore, multiple studies worldwide have exhibited that the sensitivity of AFB testing using SI varies widely, ranging from 19% to 90% [6-8, 27-29]. The sensitivity of SI AFB testing in our study was lower than in other studies. We have several hypotheses to explain this discrepancy. Firstly, the sensitivity of SI AFB testing is possibly related to the severity of the disease, the dissemination of *Mycobacteria tuberculosis*, and the number of bacteria in the specimen. Additionally, the capacity to detect *Mycobacteria tuberculosis* through AFB testing may be enhanced in cases where cavities are observed on chest imaging and may be lower in human immunodeficiency virus-infected patients [30] or in patients with miliary lung tuberculosis [31]. However, this information was not gathered in our study. Secondly, the number of sputum samples collected correlates to the test's sensitivity. Al Zahrani's study showed that the diagnostic performance of SI with one sample was 64%, two were 81%, three were 91%, and four were 98% [10]. Thirdly, our study only recruited patients with initial negative AFB results from spontaneous sputum or gastric lavage at the outpatient clinic. This may have led to the low level of *Mycobacteria tuberculosis* in the specimens and lower sensitivity of AFB SI. However, we still detected an additional 16% of patients with pulmonary tuberculosis who did not require FOB. Therefore, SI could be a method that should be considered to reduce healthcare costs and burdens in higher-level hospitals in low- to middle-income countries.

SI is a valuable method for collecting respiratory specimens, but it has certain limitations that should be considered. Patient cooperation is crucial for successful SI, as it relies on active participation and the ability to produce sputum following instructions. Additionally, resource requirements, including specific equipment and trained personnel, can pose challenges in resource-limited settings. While generally safe, sputum induction carries a small risk of complications such as bronchospasm, cough exacerbation, or respiratory distress, particularly in patients with underlying respiratory conditions. False-negative results may occur, especially in cases of paucibacillary tuberculosis, where bacterial loads in the sputum are low. Therefore, it is important to consider these limitations and employ complementary diagnostic approaches as needed.

The study has several limitations, such as not conducting MGIT culture in the SI samples, which restricts the significance of this method. Furthermore, this was a single-center study with a small sample size, making it challenging to represent the entire population with suspected pulmonary tuberculosis in the community.

Conclusion

It is strongly recommended to utilize sputum induction in clinical patients as it has been successfully employed in renowned hospitals like Cho Ray Hospital and other medical facilities in Vietnam. Sputum induction offers a safe, minimally invasive, and highly efficient approach to obtaining respiratory specimens, even from individuals who are unable to produce a spontaneous sputum sample. This method is particularly beneficial for hospitals with limited resources in developing countries. It is advisable to reserve the use of more resource-intensive procedures such as flexible fiberoptic bronchoscopy for suspected cases of pulmonary tuberculosis that yield negative results in acid-fast bacilli tests conducted on spontaneous sputum, induced sputum, and gastric lavage samples.

LIST OF ABBREVIATIONS

TB - tuberculosis; SD - standard deviation; AFB - acid-fast bacillus; BAL - bronchoalveolar lavage; MGIT - mycobacteria growth indicator tube; CTD-ILD - connective tissue disease-associated interstitial lung disease; FOB - flexible fiberoptic bronchoscopy; SI - sputum induction; FEV_1 - forced expiratory volume in one second.

ETHICAL STATEMENT

The present study obtained permission from the Ethical Review Committee, University of Medicine and Pharmacy at Ho Chi Minh City (No. 557/HĐĐĐ-ĐHYD). All patients participating in the study signed informed consent.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTION

Conceptualization, writing original protocol: N.M.D. and K.D.N.; investigation writing – original draft, and collecting data: N.M.D., K.D.N. and V.T.L.; protocol review: N.M.D.; formal analysis: N.N.T.; writing – review and editing: N.M.D., K.D.N., V.T.L. and N.N.T.. All authors, including N.M.D., K.D.N., V.T.L. and N.N.T. revised the manuscript and agreed to the final version before submission.

ORCID ID

Ngoc Minh Duong https://orcid.org/0009-0000-8372-2794
Khoa Dang Nguyen https://orcid.org/0000-0003-1063-9281
Nguyen Ngoc Tran https://orcid.org/0000-0002-6059-0992
Vu Thuong Le https://orcid.org/0000-0002-2109-913X

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