Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2023 Jan 01; 11(B):150-155.
https://doi.org/10.3889/oamjms.2023.11292
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Pulmonology*

The Usefulness of Bronchoscopy in the Diagnosis of *Mycobacterium tuberculosis Complex* Species Infection

Budi Yanti[1]*, Soetjipto Soetjipto[2,3], Ni Made Mertaniasih[3,4], Susaniwati Susaniwati[5], Muhammad Amin[6]

[1]Department of Pulmonology and Respiratory Medicine, School of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [2]Department of Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia; [3]Department of Medical Biochemistry, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia; [4]Department of Clinical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia; [5]Department of Pulmonology and Respiratory Medicine, Dr Moh Soewandhi Hospital, Surabaya, Indonesia; [6]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Edited by: Ksenija Bogoeva-Kostovska
Citation: Yanti B, Soetjipto S, Mertaniasih NM, Susaniwati S, Amin M. The Usefulness of Bronchoscopy in the Diagnosis of *Mycobacterium tuberculosis Complex* Species Infection. Open Access Maced J Med Sci. 2023 Jan 01; 11(B):150-155. https://doi.org/10.3889/oamjms.2023.11292
Keywords: Bronchoscopy; *Mycobacterium tuberculosis* complex; Pulmonary tuberculosis; Bronchoalveolar lavage
***Correspondence:** Budi Yanti, Department of Pulmonology and Respiratory Medicine, School of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. E-mail: byantipulmonologis@unsyiah.ac.id
Received: 24-Nov-2022
Revised: 05-Dec-2022
Accepted: 14-Dec-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Pulmonary tuberculosis is an active chronic infection of the lungs. It is still a public health problem globally caused by the Mycobacterium tuberculosis Complex (MTBC). These species are difficult to determine only by conventional tests. The clinical manifestations are almost similar between the strains and cause diagnosis delays. Prolonged and intolerable MTBC therapy inhibits infection control.

AIM: This study aims to evaluate the usefulness of bronchoscopy in diagnosing the MTBC species infection.

METHODS: This study recruited patients with difficulty expectorating sputum. Pulmonary tuberculosis was diagnosed with the Xpert MTB/RIF assay. This study assessed sputum Acid Fast Bacilli (AFB) staining, chest X-rays with active pulmonary tuberculosis, characteristics of Bronchoalveolar lavage (BAL), and bronchoscopic findings based on the Chung classification. The BAL of polymerase chain reaction analysis using RD9 and TbD1 primers to determine MTBC species.

RESULTS: Out of the 30 cases, *M. tuberculosis* and Mycobacterium *bovis* 24 (80.0%) and 6 (20.0%) were identified in BAL fluid. There were 12 cases (40.0%) with AFB sputum test, and 25 (83.3%) of the Xpert MTB/RIF detected tuberculosis cases. All chest X-rays showed infiltrated and 22 (73.3%) pulmonary ectasis. There was a significant difference in MTBC species between sputum and BAL fluid ($p < 0.05$). The ulcerative type of bronchoscopy findings was significantly different in MTBC species ($p < 0.05$) and there was no macroscopic BAL fluid difference ($p > 0.05$).

CONCLUSIONS: Bronchoscopy is a specimen collection technique that is beneficial in determining the diagnosis of MTBC. Analysis of BAL with molecular methods contributes to identifying MTBC species quickly and accurately.

Introduction

Mycobacterium tuberculosis (MTB) is one of the *M. tuberculosis Complex* (MTBC) species that causes pulmonary tuberculosis in humans, killing over one million people each year [1], and the leading cause of death from infectious diseases globally in 2019. A quarter of the population is infected with 10 million tuberculosis (TB) cases and 1.2 million TB deaths each year. In addition, extrapulmonary TB infection occurs in more than 15% of any tissue in the body, causing TB challenging to diagnose and treat [2]. Indonesia is the number two country that contributes the most global TB incidence [3]. Due to the slow progress of TB elimination, it is estimated that the world will not be able to contain TB as a public health threat by 2035. Contributing factors include difficulty finding TB in children, all TB patients, TB drug resistance, TB prevention, and resources for TB, including research and other operational costs globally [3]. Furthermore, there is a gap between tuberculosis cases, cure rates, and success rates of treatment in Indonesia [4]. Therefore, a lot of research is required to improve the diagnosis and treatment of pulmonary TB based on identifying the MTB [5].

M. tuberculosis and *M. bovis* are members of the MTBC. Many clinical and microbiological studies have shown that MTBC species differ in virulence, clinical manifestations, and disease severity. MTBC grows more slowly and causes less severe tuberculosis. Due to its slow growth on culture assays, mycobacterium africanum (MAF) has low bacterial virulence, such as low ESAT-6 immunogenicity, and was associated with disease severity during infection [6]. De Jong *et al*., in 2010, showed that lower body mass index and moderate lung damage were associated with West African MAF Type 2. MAF isolates showed 2.52 times less sputum smear-positive than MTB, indicating that the lung damage of MTBC is lower than MTB infection [7]. It was challenging to determine the differences between these two species based on the clinical symptom. However,

both mycobacteria showed similar symptoms, such as weight loss, anorexia, and fever [8].

MTBC has a higher content of complex lipids and long-chain mycolic acids; Therefore, it requires several laboratory techniques, such as direct staining of specimens, microorganism culture, and molecular detection [9]. The World Health Organization recommended MTB identification using a liquid culture system in various countries. Unfortunately, this method takes more than 1 month [10]. The GeneXpert MTB/RIF (Xpert) assay is a rapid molecular test for diagnosing pulmonary TB and allows the simultaneous detection of MTBC [11]. However, the MTBC has multiple copies of IS6110 and IS1081 between strains, affecting the detection threshold. The variation in copy number between strains is highly dependent on the geographic location of the host. It suggested that MTBC generally could not be detected using the Xpert assay [12].

The achievement of highly qualified biological specimens is the key to successful diagnostic and therapeutic determination. Although sputum induction is frequently performed, it does not guarantee a high-volume or high-quality specimen [13]. Several studies reported the role of bronchoscopy with the bronchoalveolar lavage (BAL) technique in diagnosing pulmonary tuberculosis, and the polymerase chain reaction (PCR) method has high sensitivity and specificity results compared to other conventional methods such as sputum and culture testing [14], [15]. Bronchoscopy is frequently performed in patients with smear-negative or expectoration of sputum is difficult [13]. The Xpert MTB/RIF assay's effectiveness with BAL specimens is similar to sputum-cultured MTB [16]. Analysis BAL with molecular method significantly improves the accuracy of diagnostic and is more valuable than the conventional method [17]. The BAL fluid is a specimen that can distinguish the types of MTBC species in the lower airway [18], [19]. Therefore, this study aims to evaluate the usefulness of bronchoscopy in recognizing MTBC species infection.

Methods

Study design and participants

This research was a cross-sectional study conducted in June-October 2017. The pulmonary TB patient undergoing diagnostic assessment at Dr. Moh Soewandhie hospital, Surabaya, was invited to participate and was recruited with informed consent. In general, patients with insufficient sputum or difficulty expectorate the sputum. The protocol was approved by the Health Research Ethics Committee of Dr. Soetomo (No: 388/PANKE/KKE/V/2017). The inclusion criteria were patients with active pulmonary TB who never received treatment between 18 and 60 years, and willing to participate in bronchoscopy. The exclusion criteria were patients with HIV, diabetes mellitus, abnormal kidney function, heart disease, systemic lupus erythematosus, rheumatoid arthritis, and non-TB pulmonary disease.

Diagnosis of pulmonary tuberculosis

Diagnosis of pulmonary TB based on Xpert MTB/RIF testing (Cepheid, Sunnyvale, CA, United States, 2016). This study also assessed sputum Acid Fast Bacilli (AFB) staining and chest X-rays with active pulmonary TB. The chest X-ray characteristics were analyzed based on the typical lesions in pulmonary TB [20]. Ziehl-Neelsen AFB staining methods and interpretation using the recommendations of the International Union Against Tuberculosis [21].

Bronchoscopy procedure

Before performing a bronchoscopy, the patient had a chest X-ray and lung function tests such as spirometry or blood gas analysis and must meet certain conditions such as cardiac risk index = 1, PO2 greater than 65 mmHg without oxygen supply, shortness of breath, hemoglobin more than 10 g/dl, and is classified in the Level I of the American Society of Anesthesiologists 1. The patients were required to receive oral anesthesia with 5 ml lidocaine and 10% xylocaine spray on the oropharynx, laryngopharynx, and vocal cords, and had to fast for 5 h before the procedure to avoid irritation of the airways and reduce airway reactivity. Furthermore, they were anesthetized, and the trachea and segmental bronchi were assessed through a bronchoscope. Suction is performed to remove mucus or blood in the airway [22]. The area with tuberculous lesions was examined and assessed as bronchoscopic findings based on the Chung classification [23]. The area was rinsed with 10 ml of 0.9% NaCl, then sucked again. This action was repeated several times until the BAL fluid is approximately ± 50 ml [24].

Identification of Mycobacterium tuberculosis complex species

The BAL fluid was centrifuged at 3000 rpm for 15–20 min, and the supernatant was transferred into another tube. The DNA extraction was conducted using DNeasy® blood and tissue kits (Ambion, Inc., Austin, TX, USA). To identify *M. tuberculosis,* the Beijing strain was used, and the primary target of RD9 (F: 5'-GTGTAGGTCAG CCCCATCC-3 ', I: 5-CAATGTTTGTTGCGCTGC-3', R: 5'-GCTACCCTCGACCAAGTGTT-3 '). While, *M. bovis* BCG strain was used as the primary target of TBD1 (F: 5'-AGTGACTGGCCTGGTCAAAC-3 ', R: 5'-GAGCTCTGTGCGACGTTATG-3') [25]. PCR reactions were performed in Thermal Cycler PCR (BIO-RAD). The PCR assays condition were set up by denaturation (94°C, 30 s), annealing (56°C, 1 s), and

extension (72°C, 10 min) with 35 cycles. Sequencing analysis was performed by the BLAST program to determine the homology of the MTBC species.

Table 1: Patients characteristics

Characteristics	n (%)
Gender	
Female	16 (53.3)
Male	14 (46.7)
Age (years)	
18–20	4 (13.3)
21–40	16 (53.3)
41–50	6 (20.0)
> 50	4 (13.3)
AFB sputum	
Negative	17 (56.7)
Positive	13 (43.3)
Chest X-ray lesion	
Nodul	
Yes	19 (63.3)
No	11 (36.7)
Infiltrate or consolidation	
Yes	30 (100)
No	
Cavitas	
Yes	4 (13.3)
No	26 (86.7)
Ectasis	
Yes	22 (73.3)
No	8 (26.7)
Gene Xpert sputum	
Positive	22 (73.3)
Negative	8 (26.7)
Species MTBC	
M. tuberculosis	24 (80.0)
M. bovis	6 (20.0)
BAL fluid macroscopic	
Clear	26 (86.7)
Reddish	2 (6.7)
Yellowish	2 (6.7)
Bronchoscopic findings	
Hyperemic	
Yes	17 (56.7)
No	13 (43.3)
Ulcerative	
Yes	17 (56.7)
No	13 (43.3)
Fibrostenosis	
Yes	4 (13.3)
No	26 (86.7)
Edema	
Yes	13 (43.3)
No	17 (56.7)

M. tuberculosis: Mycobacterium tuberculosis, MTBC: *M. tuberculosis* complex, *M. bovis: Mycobacterium bovis*, AFB: Acid fast bacilli, BAL: Bronchoalveolar lavage.

Statistical analysis

A univariate analysis was used to assess the potential variables of patients characteristics, and Fisher test was used to determine the differences between MTBC species. $p < 0.05$ was considered statistically significant. All statistical calculations were performed using the Social Sciences 18.0 software (IBM Inc., Chicago, IL, USA).

Results

Characteristics patient

Out of the 30 new TB cases that participated, the majority of the patients were women (56.7%) (Table 1). There were 12 cases (40.0%) with AFB sputum positive, and the Xpert MTB/RIF detect 25 (83.3%) MTB cases and 5 (16.7%) negative MTB cases. Identification of MTBC species by BAL fluid of PCR analysis showed 24 (80.0%) *M. tuberculosis* Beijing strain and 6 (20.0%) *M. bovis* BCG strain. All patients with infiltrated and 22 (73.3%) pulmonary ectasis based on Chest X-Rays imaging. The macroscopic of BAL fluid showed 26 (86.7%) clearly, 2 (6.7%) cloudy, and 2 (6.7%) redness. The hyperemic and edema types were bronchoscopic findings that were detected frequently in 17 (56.7%).

Comparison of Mycobacterium tuberculosis complex infection

There was no significant difference in MTBC species between AFB sputum and BAL fluid. Among MTB detected by Xpert MTB/RIF sputum, there were significantly 16 (72.7%) *M. tuberculos*is Beijing strain and 6 (27.3%) *M. bovis* BCG strains based on BAL fluid of PCR analysis ($p < 0.05$) (Table 2).

Table 2: Comparison of Mycobacterium tuberculosis complex identification based on specimens

Variable	BAL fluid		p-value
	M. tuberculosis, n (%)	*M. bovis*, n (%)	
AFB sputum			
Positive	11 (84.6)	2 (15.4)	0.580
Negative	13 (76.5)	4 (23.5)	
Gene Xpert sputum			
MTB detected	16 (72.7)	6 (27.3)	0.009
MTB not detected	8 (100)	0	

M. tuberculosis: Mycobacterium tuberculosis, MTB: *M. tuberculosis*, *M. bovis*: *Mycobacterium bovis*, BAL: Bronchoalveolar lavage, AFB: Acid fast bacilli.

Bronchoscopic findings in Mycobacterium tuberculosis complex infection

Bronchoscopic with fibrostenosis type lesion was only detected in *M. tuberculosis* strain Beijing infection. Bronchoscopy with ulcerative-type lesions is mainly seen in *M. tuberculosis* strain Beijing infection. There was a significant difference between MTBC infection in the ulcerative type lesion ($p < 0.05$) (Table 3 and Figure 1).

Discussion

AFB sputum staining may not always reveal the specific causative pathogens. Similarly, sputum culture does not always grow bacteria and it takes a long time to get a specific agent. However, this can automatically slow down the diagnosis of pulmonary TB [15].

Table 3: Bronchoscopic findings in *Mycobacterium tuberculosis* complex species infection

Bronchoscopic findings	*M. tuberculosis* complex species		p-value
	M. tuberculosis, n (%)	*M. bovis*, n (%)	
Hyperemic			
Yes	12 (70.60)	5 (29.40)	0.196
No	12 (92.30)	1 (7.70)	
Ulcerative			
Yes	14 (82.40)	3 (17.60)	0.01
No	10 (76.90)	3 (23.10)	
Fibrostenosis			
Yes	4 (100.00)	0	0.557
No	20 (76.90)	6 (23.10)	
Edema			
Yes	11 (84.60)	2 (15.40)	0.672
No	13 (76.50)	4 (23.50)	

M. tuberculosis: Mycobacterium tuberculosis, M. bovis: Mycobacterium bovis.

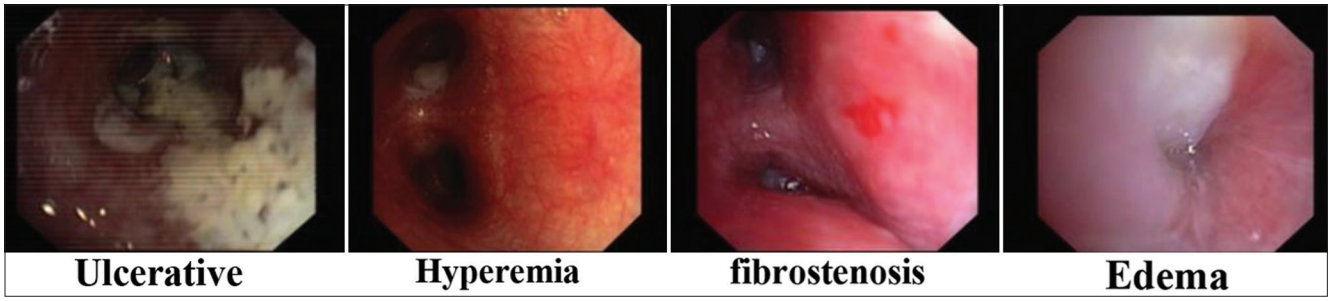

Figure 1. Bronchoscopic lesion findings

This study showed that MTBC species were detected in all patients with pulmonary tuberculosis based on BAL fluid from the PCR analysis (Table 1). In contrast, the Xpert MTB/RIF sputum test showed that only 22 (73.3%) MTB were detected. High-quality specimens can be obtained by bronchoscopy technique in patients who cannot cough up sputum or patients with negative sputum results. Two main bronchoscopy techniques achieve high-quality specimens: BAL and bronchial washing [15]. Therefore, bronchoscopy using BAL fluid of PCR analysis is very useful in determining the pathogen that causes the disease specifically and accurately. In addition, BAL fluids not only improve the ability of bacteriological detection for diagnosis but can increase the precision of effective treatment through MTBC species identification and drug resistance testing [26], [27], [28].

The evaluation of bronchoscopic findings was performed based on Chung's classification and different types of bronchoscopy occurred in 30 patients. The initial microscopic type was submucosal lymphocytic infiltration which was also common in non-specific bronchitis [23]. The formation of submucosal tubercles is a development of the previous type and will appear in bronchoscopy as a granular type and an edematous-hyperemic type. The ulceration type occurs in erosion on the mucosal surface and develops into a small lump that leads to hyperplastic activity. It is known as a type of fibrostenosis [29]. Furthermore, this study showed that hyperemic, edema, ulcerative, and fibrostenosis types were mostly presented in *M. tuberculosis* strain Beijing than *M. bovis* strain BCG infection (Table 3). This may be due to differences in virulence between MTBC species and *M. tuberculosis* Beijing strain has high virulence such as the ability to induce lung parenchymal severe damage with extensive necrotizing pneumonia [30], [31]. An *in vitro* study showed that there are differences in the ability to induce pulmonary cell death and to release various cellular components as harmful signals in macrophages infected by the *M tuberculosis* Beijing strain [32].

An additional test was required to improve the diagnostic value of bronchoscopy, and the intervention achieved the diagnostic value of 1.5%–33.8% by performing the PCR method or cytological analysis of BAL fluid [33]. Furthermore, bronchoscopy also improves the diagnostic value (26%–68%) after performing additional diagnostic test such as microbiological culture and histopathological analysis [34]. Similar to others, this study assumes that bronchoscopy with PCR analysis is a very useful tool in diagnosing pulmonary tuberculosis, malignancy, hemoptysis, and history of excessive smoking, especially when taking samples for specific diagnosis such as BAL fluids, biopsy, and cytology [35].

Conclusion

Bronchoscopy with BAL is a collection of high-quality specimens and can provide visualization of the airways to identify mucosal lesions. In addition, BAL analysis with molecular methods contributes to the rapid and accurate identification of MTBC species, therefore contributing to definitively diagnosing MTBC infection. The ulcerative type of bronchoscopic finding was observed in the *M. tuberculosis* Beijing strain infection.

Acknowledgement

The authors thank to the Electron Microscope Laboratory and Clinical Pathology Department for all support on this study.

Author's contribution

Handling manuscript, data collection: BY, S, and NMM. Review and improvement: BY, S, NMM, S, and MA. All authors participated in the manuscript's preparation and agreed on the final version.

References

1. World Health Organization. Are Updated Every Year for the Tuberculosis. Geneva: World Health Organization; 2020. Available from: https://www.who.int/tb/publications/global_report/en [Last accessed on 2022 Apr 15].
2. Moule MG, Cirillo JD. *Mycobacterium tuberculosis* Dissemination plays a critical role in pathogenesis. Front Cell Infect Microbiol. 2020;10:65. https://doi.org/10.3389/fcimb.2020.00065
   PMid:32161724
3. Chakaya J, Khan M, Ntoumi F, Aklillu E, Fatima R, Mwaba P, *et al*. Global tuberculosis report 2020 reflections on the global TB burden, treatment and prevention efforts. Int J Infect Dis. 2021;1(1):S7-12. https:/doi.org/10.1016/j.ijid.2021.02.107
   PMid:33716195
4. Ahmad RA, Matthys F, Dwihardiani B, Rintiswati N, De Vlas SJ, Mahendradhata Y, *et al*. Diagnostic work-up and loss of tuberculosis suspects in Jogjakarta, Indonesia. BMC Public Health. 2012;12(1):132. https://doi.org/10.1186/1471-2458-12-132
   PMid:22333111
5. Yanti B, Mulyadi M, Amin M, Harapan H, Mertaniasih NM, Soetjipto S. The role of *Mycobacterium tuberculosis* complex species on apoptosis and necroptosis state of macrophages derived from active pulmonary tuberculosis patients. BMC Res Notes. 2020;13(1):415. https://doi.org/10.1186/s13104-020-05256-2
   PMid:32887662
6. Baya B, Diarra B, Diabate S, Kone B, Goita D, Sarro S, *et al*. Association of *Mycobacterium africanum* infection with slower disease progression compared with *Mycobacterium tuberculosis* in Malian patients with tuberculosis. Am J Trop Med Hyg. 2020;102(1):36-41. https: doi.org/10.4269/ajtmh.19-0264
   PMid:31733052
7. De Jong BC Antonio M, Gagneux S. *Mycobacterium africanum* review of an important cause of human tuberculosis in West Africa. PLoS Negl Trop Dis. 2010;4(9):e744. https://doi.org/10.1371/journal.pntd.0000744
   PMid:20927191
8. Forrellad MA, Klepp LI, Gioffré A, García JS, Morbidoni HR, Santangelo M, *et al*. Virulence factors of the *Mycobacterium tuberculosis* complex. Virulence. 2013;4(1):3-66. https://doi.org/10.4161/viru.22329
   PMid:23076359
9. Caulfield AJ, Wengenack NL. Diagnosis of active tuberculosis disease : From microscopy to molecular techniques. J Clin Tuberc Other Mycobact Dis. 2016;4:33-43. https://doi.org/10.1016/j.jctube.2016.05.005
   PMid:31723686
10. Mokaddas E, Ahmad S, Eldeen H. Performance comparison of geneXpert MTB/RIF and probetec ET tests for rapid molecular diagnosis of extrapulmonary tuberculosis in a Low TB/MDR-TB incidence country. Med Princ Pract. 2021;30(3):277-84. https://doi.org/10.1159/000515254
    PMid:33592621
11. Shen G, Chiou C, Hu ST, Wu KM, Chen JH. Rapid identification of the *Mycobacterium tuberculosis* complex by combining the ESAT-6/CFP-10 immunochromatographic assay and smear morphology. J Clin Microbiol. 2011;49(3):902-7. https://doi.org10.1128/JCM.00592-10
    PMid:21159936
12. Goosen WJ, Kerr TJ, Kleynhans L, Warren RM, Van Helden PD, Persing DH, *et al*. The Xpert MTB/RIF ultra assay detects *Mycobacterium tuberculosis* complex DNA in white rhinoceros (*Ceratotherium simum*) and African elephants (*Loxodonta africana)*. Sci Rep. 2020;10(1):14482. https://doi.org/10.1038/s41598-020-71568-9
    PMid:32879401
13. Theron G, Peter J, Meldau R, Khalfey H, Gina P, Matinyena B, *et al*. Accuracy and impact of Xpert MTB/RIF for the diagnosis of smear-negative or sputum-scarce tuberculosis using bronchoalveolar lavage fluid. Thorax. 2013;68(11):1043-51. https://doi.org/10.1136/thoraxjnl-2013-203485
    PMid:23811536
14. Ahmad M, Ibrahim WH, Sarafandi SA, Shahzada KS, Ahmed S, Haq IU, *et al*. Diagnostic value of bronchoalveolar lavage in the subset of patients with negative sputum/smear and mycobacterial culture and a suspicion of pulmonary tuberculosis. Int J Infect Dis. 2019;82:96-101. https://doi.org/10.1016/j.ijid.2019.03.021
    PMid:30904678
15. Kim YW, Kwon BS, Lim SY, Lee YJ, Cho YJ, Yoon HI, *et al*. Diagnostic value of bronchoalveolar lavage and bronchial washing in sputum-scarce or smear-negative cases with suspected pulmonary tuberculosis : A randomized study. Clin Microbiol Infect. 2020;26(7):911-6. https://doi.org/10.1016/j.cmi.2019.11.013
    PMid:31759097
16. Sun Y, Zhang Q, Zhang Q, Liu C, Zhang H, Fu Y. Diagnostic efficacy of xpert MTB/RIF assay in bronchoalveolar lavage fluid for tracheobronchial tuberculosis : A retrospective analysis. Front Med. 2021;8:682107. https://doi.org/10.3389/fmed.2021.682107
    PMid:34485328
17. Yan L, Zhang Q, Xiao H. Clinical diagnostic value of simultaneous amplification and testing for the diagnosis of sputum-scarce pulmonary tuberculosis. BMC Infect Dis. 2017;17(545):1-6. https://doi.org/10.1186/s12879-017-2647-7
    PMid:28779754
18. Güven S, Yilmaz E, Kutbay H, Sariyildiz S, Dalar L, Poluman A. The diagnostic value of polymerase chain reaction (PCR) in bronchioalveolar lavage. East J Med 2004;9(1):7-12.
19. Jarvela J, Moyer M, Leahy P, Bonfield T, Fletcher D, Mkono WN, *et al*. *Mycobacterium tuberculosis*-induced bronchoalveolar lavage gene expression signature in latent tuberculosis infection is dominated by pleiotropic effects of $CD4^+$ T cell-dependent IFN-γ production despite the presence of polyfunctional t cells within the. J Immunol. 2019;203(8):2194-209. https://doi.org/10.4049/jimmunol.1900230
    PMid:31541022
20. Kurashima, Kozo MA. A method for visual scoring of pulmonary *Mycobacterium avium* complex disease: NICE scoring system. Mycobacteriol Dis. 2013;3(2):3-7.
21. Rieder HL, Van Deun A, Kam KM, Kim SJ, Chonde TM, Trébucq A, *et al*. Priorities for Tuberculosis Bacteriology Services in Low-Income Countries. 2nd ed. Paris: International Union Against Tuberculosis and Lung Disease; 2007. Available from: https://www.tbrieder.org/publications/books_english/red_book.pdf [Last accessed on 2022 May 11].
22. Du Rand IA, Blaikley J, Booton R, Chaudhuri N, Gupta V, Khalid S, *et al*. British thoracic society guideline for diagnostic flexible bronchoscopy in adults. Thorax 2013;68(Suppl 1):i1-44. https://doi.org/10.1136/thoraxjnl-2013-203618
    PMid:23860341
23. Chung HS, Lee JH. Bronchoscopic assessment of the evolution of endobronchial tuberculosis. Chest. 2000;117(2):385-92. https://doi.org/10.1378/chest.117.2.385
    PMid:10669679
24. Baughman RP. Technical aspects of bronchoalveolar lavage: Recommendations for a standard procedure.

Semin Respir Crit Care Med. 2007;28(5):475-85. https://doi.org/10.1055/s-2007-991520

PMid:17975775

25. Parsons LM, Brosch R, Cole ST, Somoskövi Á, Loder A, Bretzel G, *et al*. Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis. J Clin Microbiol. 2002;40(7):2339- 45. https://doi.org/10.1128/JCM.40.7.2339-2345.2002

PMid:12089245

26. Neves CP, Costa AG, Safe IP, Brito AD, Jesus JS, Kritski AL, *et al*. The role of mini-bronchoalveolar lavage fluid in the diagnosis of pulmonary tuberculosis in critically ill patients. BMC Infect Dis. 2020;20(1):299. https://doi.org/10.1186/s12879-020-04954-3

PMid:32188399

27. Chen NH, Liu YC, Tsao TC, Wu TL, Hsieh MJ, Chuang ML, *et al*. Combined bronchoalveolar lavage and polymerase chain reaction in the diagnosis of pulmonary tuberculosis in smear-negative patients. Int J Tuberc Lung Dis. 2002;6(4):350-5.

PMid:11936745

28. Liu X, Hou XF, Gao L, Deng GF, Zhang MX, Deng QY, *et al*. Indicators for prediction of *Mycobacterium tuberculosis* positivity detected with bronchoalveolar lavage fluid. Infect Dis Poverty. 2018;7(1):22. https://doi.org/10.1186/s40249-018-0403-x

PMid:29580276

29. Morrone N, Abe NS. Bronchoscopic findings in patients with pulmonary tuberculosis. J Bronchol Interv Pulmonol. 2007;14(1):15-8. https://doi.org/10.1097/LBR.0b013e31802c2fcb

30. Ribeiro SC, Gomes LL, Amaral EP, Andrade MR, Almeida FM, Rezende AL, *et al*. *Mycobacterium Tuberculosis* strains of the modern sublineage of the beijing family are more likely to display increased virulence than strains of the ancient sublineage. J Clin Microbiol. 2014;52(7):2615-24. https://doi.org/10.1128/JCM.00498-14

PMid:24829250

31. Amin M, Yanti B, Harapan H, Mertaniasih NM. The role of *Mycobacterium tuberculosis* lineages on lung tissue damage and TNF-α level among tuberculosis patients, Indonesia. Clin Epidemiol Glob Health. 2019;7(3):263-7. https://doi.org/10.1016/j.cegh.2018.11.002

32. Portevin D, Gagneux S, Comas I, Young D. Human macrophage responses to clinical isolates from the *Mycobacterium tuberculosis* complex discriminate between ancient and modern lineages. PLoS Pathog. 2011;7(3):e1001307. https://doi.org/10.1371/journal.p

PMid:21408618

33. Khan DF, Suleman M, Baijnath P, Perumal R, Moodley V, Mhlane Z, *et al*. Multiple microbiologic tests for tuberculosis improve diagnostic yield of bronchoscopy in medically complex patients. AAS Open Res. 2019;2:25. https://doi.org/10.12688/aasopenres.12980.1

PMid:32382702

34. Heching M, Rosengarten D, Shitenberg D, Shtraichman O, Abdel-Rahman N, Unterman A, *et al*. Bronchoscopy for chronic unexplained cough: Use of biopsies and cultures increase diagnostic yield. J Bronchology Interv Pulmonol. 2020;27(1):30-5. https://doi.org/10.1097/LBR.0000000000000629

PMid:31651543

35. Gasparini S. Indications for diagnostic bronchoscopy in adults. Monaldi Arch Chest Dis. 2011;75(1):24-31. https://doi.org/10.4081/monaldi.2011.236

PMid:21626989