European Review for Medical and Pharmacological Sciences 2022; 26: 1738-1742

Microbiology rapid on-site evaluation: a better method for Mucoid Pseudomonas Aeruginosa diagnosis in bronchiectasic patients

T. LI[1], Y.-T. HUO[1], X.-Q. ZHENG[1], M.-S. FANG[1], G.-L. QUAN[1], G. XIAO[2], Y.-X. CHENG[1]

[1]Department of Respiratory Medicine, The Third Affliated Hospital of Southern Medical University, Guangzhou, China
[2]Department of Clinical Laboratory, The Third Affliated Hospital of Southern Medical University, Guangzhou, China

Ting Li and Yating Huo contributed equally to this work as first authors

Abstract. – OBJECTIVE: The aim of this study is to find an accurate and fast method to diagnose the pathogen of bronchiectasis.

PATIENTS AND METHODS: Ten bronchiectasic patients diagnosed with Mucoid Pseudomonas Aeruginosa (MPA) in the past two years were analyzed. Accuracy and time were compared between microbiology rapid on-site evaluation (M-ROSE) and sputum bacterial culture.

RESULTS: The accuracy rate of M-ROSE in the patients is 100% consistent with bacterial culture results. The average time of M-ROSE is about 4.3 min, which is over 1000 times shorter than that of sputum bacterial culture.

CONCLUSIONS: M-ROSE may be a better method for etiological diagnosis of MPA.

Key Words:
Bronchiectasis, Microbiology rapid on-site evaluation, Mucoid *pseudomonas aeruginosa*.

Introduction

Bronchiectasis is an irreversible airway dilation that involves the lung in either focal or diffused manner. The epidemiology of bronchiectasis reached 566 every 100 thousand, which increased by 40% in the past 10 years[1]. Chronic infection is one of the characteristics of bronchiectasis. About 80% of bronchiectasic patients can be found with pathogens in the sputum[2], of which the most common species include *Pseudomonas aeruginosa* and *Haemophilus influenzae*. Repeated infection with *P. aeruginosa* may lead to increasing hospitalization frequency, decreasing life quality, and forced expiratory volume in one second (FEV_1)[3], especially Mucoid Pseudomonas Aeruginosa (MPA).

MPA is a mutation that can produce biofilms more easily and exhibits enhanced recalcitrance to antimicrobial therapy. Thus, it shows multidrug resistance. MPA can also produce biofilms, which cause repeated inflammation of bronchial mucosa. To sum up, MPA is associated with a worse prognosis in bronchiectasis[4], a precipitous decline in pulmonary function and higher mortality. Nowadays, the diagnosis of MPA mainly depends on sputum culture, which takes quite long time. Hence, an accurate and much speedy method is needed to diagnose MPA.

Rapid on-site evaluation (ROSE), which includes cytology (C)-ROSE and microbiology (M)-ROSE, has become an indispensable part of the current respiratory interventional medicine. C-ROSE can quickly feedback the results to the physician and increase the positive rate of lung puncture biopsy[5,6]. M-ROSE is widely used in the diagnosis of pulmonary infectious diseases, and can distinguish Gram-positive bacteria, Gram-negative bacteria and fungus, especially aspergillus, monilia and *cryptococcus*[7].

In this study, we retrospectively examined 10 bronchiectasic patients diagnosed with MPA infection in the past two years. Then, the accuracy and time were compared between sputum culture and M-ROSE, aiming to find a quick and accurate method for MPA diagnosis.

Patients and Methods

Study Design and Subjects

We investigated 10 bronchiectasic patients treated in the Third Affiliated Hospital of Southern Medical University between March 2019 and Novem-

Corresponding Author: Guoli Quan, MD; e-mail: quangguoli020726@163.com; Gang Xiao, MD; e-mail: xiaogang2993@yeah.net; Yuanxiong Cheng, MD; drchengyx@163.com

Table I. Clinical statistics of patients.

Characteristic	Male	Female	Age, mean (years)	Mean course of disease (years)
	5	5	59.6±16.379	8.85±9.574

ber 2020. Bronchiectasis was diagnosed based on international guidelines. Inclusion criteria were age >18 years; confirmed diagnosis of bronchiectasis and infection by high-resolution chest computed tomography (CT); chronic cough and sputum; signed informed consent. Exclusion criteria were bronchiectasis with hemoptysis, inability to retain sputum specimens or sign an informed consent form, active tuberculosis, concurrent tumors in any organ. These bronchiectasic patients were diagnosed with MPA by both sputum culture and M-ROSE. Patients with active tuberculosis, traction bronchiectasis, malignancy, or severe systemic diseases were excluded. The demographic characteristics of the bronchiectasic patients were listed in Table I.

Tracheoscopy Protocol

Sputum was collected from the bronchiectasic patients *via* flexible fiberoptic bronchoscopy (FFB). For each subject, sputum was obtained from the dilated subsegmental airway, placed on ice and transported to the research laboratory.

Microbiological Analyses

The sputum samples were processed on-site by a trained respiratory physician. Each sample was divided into two equal parts, which were used for ROSE and bacterial culture respectively.

The sputum samples for ROSE were fixed for 10 s and stained using Diff-Quik reagents (20 to 30 s with solution A, 30 to 40 s with solution B). Rapid on-site cytology characterization was then performed using an optical microscope. Bacterial culture was conducted in the clinical laboratory.

Statistical Analysis

Data of continuous variables were presented as mean ± standard deviation (SD) and analyzed using *t*-tests. Statistical analyses were performed on SPSS 18.0 (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

Results

Morphology of MPA

Due to the mucus layer coating, MPA after staining showed a unique light purple biofilm under the microscope, which included blue-dyed Gram-negative bacilli that can be quickly identified by ROSE (Figure 1).

Accuracy of ROSE

The sputum samples of the 10 bronchiectasic patients were all found with MPA by C-ROSE, which presents the same result as bacterial culture. The accuracy rate of M-ROSE in the patients is 100% consistent with bacterial culture results, indicating that ROSE is highly accurate for MPA diagnosis.

Timeliness of ROSE

The average time to complete ROSE and bacterial culture of the patients was about 4.3 and 5046 min respectively, indicating the time to complete the diagnosis by M-ROSE is over 1000 times shorter than that of bacterial culture. Thus, M-ROSE will enable earlier intervention and reduce complications, leading to better therapeutic outcomes (Figure 2).

Discussion

M-ROSE has an equal accuracy rate and shorter detection time compared with traditional sputum culture, indicating that M-ROSE may become a better method for etiological diagnosis of lung infections.

P.aeruginosa is the most common pathogen of bronchiectasic patients. It is originally almost nonmucoid variants[8], which can change to the more drug-resistant mucoid variants[9-12]. Since MPA is associated with lower lung function and higher mortality[3,13,14], fast recognition of MPA is very important for the outcome of the disease.

ROSE was used as a respiratory interventional technique since 1981 and was not widespread as an effective auxiliary intervention and diagnosis technology until 2005 when minimally invasive surgery techniques such as transbronchial needle aspiration and lung biopsy were popularized. The use of ROSE was matured around 2010. Due to the urgent demand since 2010 for microbiological etiology results in treatment of critical respiratory diseases, ROSE becomes a

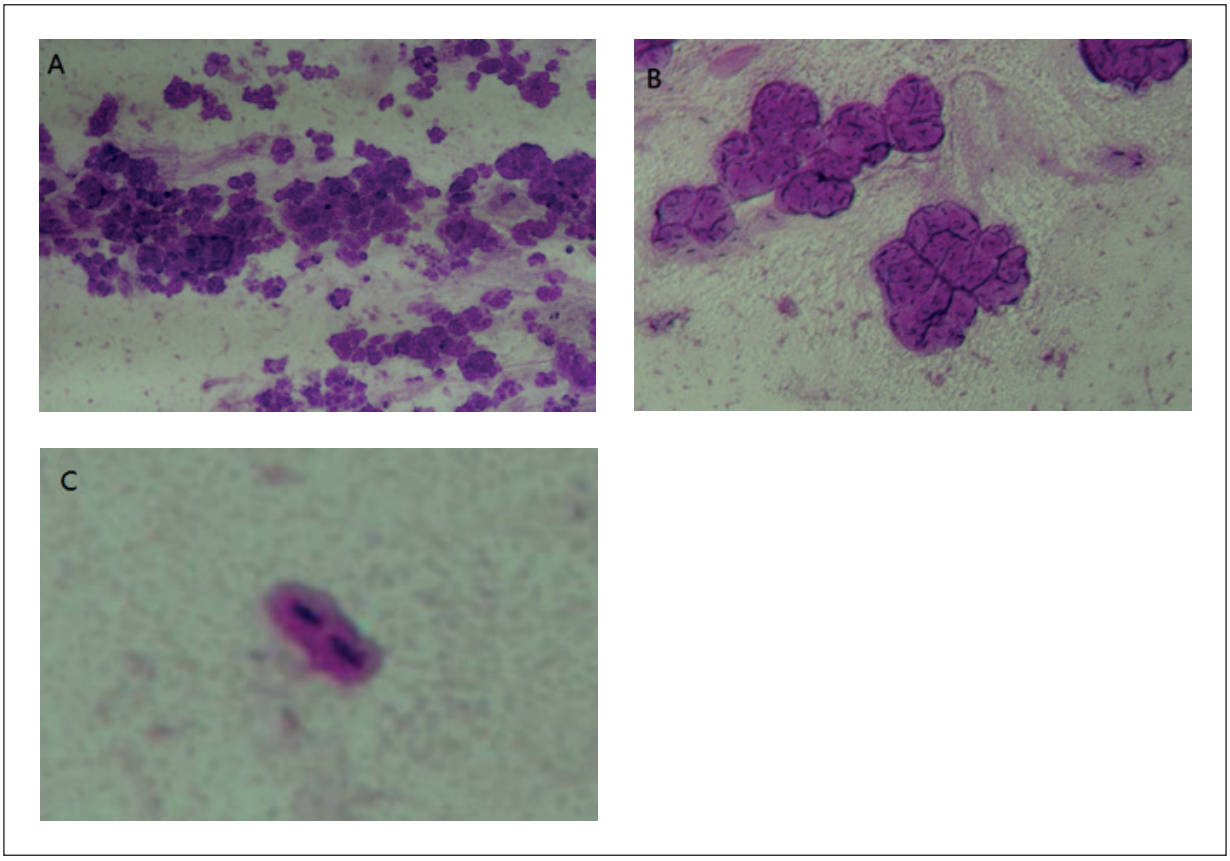

Figure 1. ROSE staining showed blue-dyed Gram-negative bacilli encased in a purple biofilm (A: X100; B: X400). Local magnification of a single thallus after ROSE staining (C: X1000).

"standard" in modern interventional pulmonology. Of the two types of ROSE, C-ROSE is mainly used for rapid tumor cell recognition, and can feedback promptly whether material collection is qualified during respiratory interventional medicine, which saves unnecessary aspiration. Moreover, M-ROSE is mainly used for cytological and microbiological identification of lung biopsy samples collected by lung puncture or bronchoscopy, so that doctors can quickly confirm the type of infection. The operational process of ROSE only includes three steps (sectioning, staining, judging), and takes about 3-5 minutes totally. At present, Diff staining is often adopted, which only needs two staining liquids (A and B), a fixation liquid, a microscope and a computer for the whole process.

With the development of respiratory intervention and rapid staining technology, ROSE becomes increasingly popular in respiratory interventional medicine. Of the two types of ROSE, C-ROSE and M-ROSE are extensively applied for diagnosis of neoplastic diseases and

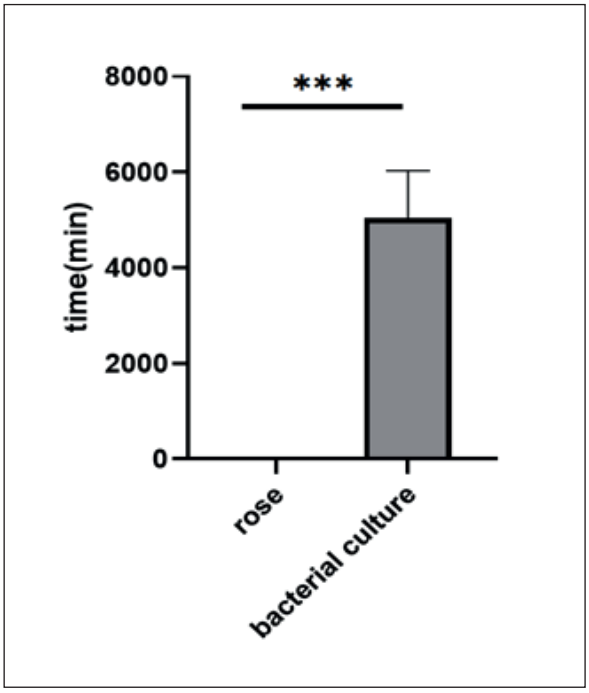

Figure 2. Time of ROSE diagnosis. *** $p < 0.001$.

lung infections respectively. The accuracy rate of M-ROSE is about 70% for tuberculosis and more than 90% for fungus infection[15], but its accuracy for bacterial infection is unknown. Our study indicates the coincidence rate between M- ROSE and pathology is about 100%. Our results demonstrate the M-ROSE results completely conform with bacterial culture. In addition to the high accuracy, M-ROSE becomes increasingly important also due to its quick turn-out time, as the time to complete the diagnosis by ROSE is over 1000 times shorter than by bacterial culture.

MPA has the special morphological characteristics that can help physicians quickly find the bacteria under microscopy. A respiratory physician trained for 3 months can give accurate results of M-ROSE at the accuracy of about 80%, which is not significantly different from the 92% of pathologists[16]. Thus, ROSE will enable earlier intervention and better therapeutic outcomes.

Conclusions

This study shows M-ROSE has quick turn-out time and high accuracy for diagnosis of MPA in bronchiectasic patients. However, since the number of patients in our study is very limited, our results need to be validated or improved in more patients in the future.

Author Contributions

Conceptualization, T.L. and Y.C.; methodology, T.L. and Y.H.; validation, X.Z. and M.F.; formal analysis, X.Z.; resources, G.Q. and G.X.; data curation, X.Z; writing– original drafting, Ting Li; writing– review and editing, all authors; visualization, X.Z. and G.X.; supervision, Y.C. and G.X.; project administration, Y.C. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

This retrospective study was conducted at a single academic medical center and was waived for institutional review board.

Informed Consent Statement

The informed consent requirement was waived. Patient consent was waived due to this retrospective analysis.

Data Availability Statement

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1) Quint JK, Millett ER, Joshi M, Navaratnam V, Thomas SL, Hurst JR, Smeeth L, Brown JS. Changes in the incidence, prevalence and mortality of bronchiectasis in the UK from 2004 to 2013: a population-based cohort study. Eur Respir J 2016; 47: 186-193.
2) Ishak A, Everard ML. Persistent and Recurrent Bacterial Bronchitis-A Paradigm Shift in Our Understanding of Chronic Respiratory Disease. Front Pediatric 2017; 5: 19.
3) Langan KM, Kotsimbos T, Peleg AY. Managing Pseudomonas aeruginosa respiratory infections in cystic fibrosis. Curr Opin Infect Dis 2015; 28: 547-556.
4) Weiss ST, Colin AA, Levy H, Kalish LA, Cannon CL, Garcı´a KC, Gerard C, Goldmann D, Pier GB. Predictors of mucoid Pseudomonas colonization in cystic fibrosis patients. Pediatr Pulmonol 2008; 43: 463-471.
5) Collins BT, Chen AC, Wang JF, Bernadt CT, Sanati S. Improved laboratory resource utilization and patient care with the use of rapid onsite evaluation for endobronchial ultrasound fine-needle aspiration biopsy. Cancer Cytopathol 2013; 121: 544-551.
6) Hongbo G, Sujing L, Jun G, Bobo L, Wanhu L, Zuowei L, Jujie S, Baijiang Z, Jinming Y. Rapid on-site evaluation during endo-bronchial ultrasound-guided transbronchial needle aspiration for the diagnosis of hilar and mediastinal lymphadenopathy in patients with lung cancer. Cancer Lett 2016; 371: 182-186.
7) Zhang X, Ye L, Hong L. Rapid on-site evaluation of pulmonary cryptococcosis: a preliminary Assessment. Asian J Surg 2020; 43: 1101-1102.
8) Govan JR, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid Pseudomonas aeruginosa and Burkholderia cepacia. Microbiol Rev 1996; 60: 539-574.
9) Hodges NA, Gordon CA. Protection of Pseudomonas aeruginosa against Ciprofloxacin and beta-lactams by Homologous Alginate. Antimicrob Agents Chemother 1991; 35: 2450-2452.
10) Hentzer M, Teitzel GM, Balzer GJ, Heydorn A, Molin S, Givskov M, Parsek MR. Alginate Overproduction Affects Pseudomonas aeruginosa Biofilm Structure and Function Alginate Overproduction Affects Pseudomonas aeruginosa Biofilm Structure and Function. J Bacteriol 2001; 183: 5395-5401.
11) Goltermann L, Tolker-Nielsen T. Importance of the exopolysaccharide matrix in antimicrobial tol-

erance of Pseudomonas aeruginosa aggregates. Antimicrob Agents Chemother 2017; 61: e02696-16.
12) Hengzhuang W, Wu H, Ciofu O, Song Z, Høiby N. Pharmacokinetics/pharmacodynamics of colistin and imipenem on mucoid and nonmucoid Pseudomonas aeruginosa biofilms. Antimicrob Agents Chemother 2011; 55: 4469-4474.
13) Li Z, Kosorok MR, Farrell PM, Laxova A, West SE, Green CG, Collins J, Rock MJ, Splaingard ML. Longitudinal development of mucoid Pseudomonas aeruginosa infection and lung disease progression in children with cystic fibrosis. JAMA 2005; 293: 581-588.
14) Farrell PM, Collins J, Broderick LS, Rock MJ, Li Z, Kosorok MR, Laxova A, Gershan WM, Brody AS. Association between Mucoid Pseudomonas Infection and Bronchiectasis in Children with Cystic Fibrosis. Radiology 2009; 252: 534-543.
15) Jing F, Guowu Z, Wen L, Chen M, Hongmei Z, Caili L, Jie C. The standard operating techniques for diagnostic interventional pulmonology based on rapid on-site evaluation. Tianjin Med J 2017; 45: 638-642.
16) Bonifazi M, Sediari M, Ferretti M, Poidomani G, Tramacere I, Mei F, Zuccatosta L, Gasparini S. The role of the pulmonologist in rapid on-site cytologic evaluation of transbronchial needle aspiration : a prospective study. Chest 2014; 145: 60-65.