Int J Clin Exp Med 2020;13(10):8027-8032
www.ijcem.com /ISSN:1940-5901/IJCEM0115173

Original Article

Evaluation of assays on bronchoalveolar lavage fluid in early diagnosis of pulmonary tuberculosis

Na Ye[1], Teer Ba[2], Yongli Yang[1], Faman Yang[1], Yuanjun Wang[1], Mei Qi[1], Xiaolong Ye[1], Yiqing Luo[3]

[1]General Practice, Affiliated Hospital of Qinghai University, Xining 811600, Qinghai Province, China; [2]General Practice, People's Hospital of Haixi, Delingha 817000, Qinghai Province, China; [3]Ophthalmology Department, Affiliated Hospital of Qinghai University, Xining 811600, Qinghai Province, China

Received May 28, 2020; Accepted July 22, 2020; Epub October 15, 2020; Published October 30, 2020

Abstract: Objective: To explore the performance of different determination assays on bronchoalveolar lavage fluid (BALF) in the early diagnosis of pulmonary tuberculosis. Methods: From July 2017 to June 2019, 120 patients with pulmonary tuberculosis (including 60 cases of sputum smear-negative and 60 cases of sputum smear-positive) treated in our hospital and 60 patients with nontuberculous mycobacterial (NTM) pulmonary disease were enrolled. All patients underwent bronchoscopy, and BALF of all patients was tested by smear method for acid-fast bacilli, colloidal gold method for tuberculosis antibodies and fluorescent quantitative PCR (FQ-PCR) method for tuberculosis DNA. The diagnostic results were used as the gold standard to compare the diagnostic efficacy of different detection methods and record the occurrence of adverse reactions. Results: The sensitivity, specificity, and accuracy of FQ-PCR and culture method in diagnosis of tuberculosis were significantly higher than those of colloidal gold method and smear method ($P<0.05$). The sensitivity, specificity and accuracy of colloidal gold method + smear method were significantly lower than those of FQ-PCR + culture method, FQ-PCR + colloidal gold method, FQ-PCR + smear method, culture method + colloidal gold method, and culture method + smear method, respectively ($P<0.05$), of which FQ-PCR + culture method had the highest sensitivity of 98.33%, specificity of 90.00%, and accuracy of 94.17%, respectively. Conclusion: FQ-PCR method and culture method are helpful to determine the condition of patients with tuberculosis in the early stage, and the combination of the two detection methods can help improve the early diagnosis rate of tuberculosis.

Keywords: Tuberculosis, early diagnosis, alveolar lavage fluid, smear method, colloidal gold method, fluorescence quantification, bacterial culture

Introduction

Pulmonary tuberculosis is a clinically common chronic infectious disease caused by *Mycobacterium tuberculosis*, which can invade multiple organs and mainly attack the lungs. If not treated in time, it will damage the patient's respiratory system, leading to life-threatening [1]. Currently, there are various methods for clinical detection of *Mycobacterium tuberculosis*, but all of them are based on bacteriology. Among them, the positive rate of smear detection for *Mycobacterium tuberculosis* is low, and the bacterial culture method takes a long time. Meanwhile, the false positive rate of polymerase chain reaction (PCR) detection was also very high, leading to clinical misdiagnosis and failure in timely diagnosis of tuberculosis [2-4]. Therefore, it is of great clinical significance to actively search for a rapid and efficient diagnostic scheme for patients with pulmonary tuberculosis. In recent years, with the continuous progress of medical technology, the fiberoptic bronchoscopy has been widely used in the diagnosis and treatment of some diseases. Through the fiber bronchial examination, the bronchoalveolar lavage fluid (BALF) was obtained to determine the specimen concentration of *Mycobacterium tuberculosis* [5-8]. In this study, smear method, colloidal gold method, cultured method and fluorescent quantitative PCR (FQ-PCR) method were used to detect BALF of all the subjects, and the detection results of different detection methods were compared,

aiming to provide reference for clinical diagnosis of tuberculosis more accurately.

Materials and methods

Baseline data

From July 2017 to June 2019, 120 patients with pulmonary tuberculosis (including 60 cases of sputum smear-negative and 60 cases of sputum smear-positive) treated in our hospital and 60 patients with nontuberculous mycobacterial (NTM) pulmonary disease were enrolled as the subjects of the study. All patients with pulmonary tuberculosis met the standards of "Guidelines for the Diagnosis and Treatment of Tuberculosis" [9]. Patients who had previously received antituberculosis treatment, or those who had previously received antituberculosis treatment before admission, or those who were infected with HIV, or those who had blood transfusion 1 week before admission were excluded. This study has been approved by the Ethics Committee of Affiliated Hospital of Qinghai University. All study participants provided written informed consent before participating in the study.

Methods

All patients underwent bronchoscopy. According to the results of chest CT or chest radiograph, the affected bronchopulmonary segments were lavaged, that is, 10-20 mL of saline was infused into the corresponding bronchopulmonary segments through fiberoptic bronchoscopy. BALF was collected into a sterile bottle with negative pressure. 20 mL of BALF should be collected for lab testing. (1) Smear method and culture method: After all BALF specimens were processed in Roche Culture Tube, the detection was carried out in strict accordance with standardized procedures. (2) Colloidal gold method: After all BALF specimens were processed, the inspection should be carried out in strict accordance with the standardized procedures of Tuberculosis Bacteriology in China. (3) Real-time fluorescence PCR: 1-1.5 mL of BALF was added to a centrifuge, centrifuged at 12000 r/min for 5 min. The supernatant was discarded, and then 1 mL of sterile saline was added and shaken thoroughly to ensure mixing. Subsequently, the tube was centrifuged at 12000 r/min for 5 min. The supernatant was discarded again, and then 0.5 μL of DNA extract was added to the precipitate for thorough shaking. The tube was stored in a 100°C thermostat for 10 min, and then put in a 4°C refrigerator for cooling. After the temperature reached room temperature, the tube was centrifuged at 12000 r/min for 5 min. 2 μL of supernatant was added to the PCR reaction tube, centrifuged at 8000 r/min for 20 s, and then placed on the PCR instrument. The amplification was performed with the following conditions: 93°C, 2 min pre-denaturation; 10 cycles of 93°C, 45 s, 55°C, 60 s; 30 cycles of 93°C, 45 s, and 55°C, 120 s. After the completion of PCR reaction, the relative expression was detected by fluorescence; the minimum sensitivity was obtained from 50 copies.

Observation outcomes

The results of different assays for *Mycobacterium tuberculosis* were compared and the adverse reactions were recorded in all patients.

Statistical analysis

SPSS 20.0 was used for data processing. The count data were expressed as a percentage and compared using χ^2 test. $P<0.05$ was considered statistically significant.

Results

General information

Among the 120 pulmonary tuberculosis patients, there were 65 males and 55 females, aged 23-75 years, with the average age of (58.73±12.66) years. NTM pulmonary disease patients included 32 males and 28 females, aged 22-77 years, with the average age of (58.35±11.80) years. There were 23 cases of pulmonary inflammation, 17 cases of bronchial foreign body, 13 cases of bronchiectasis, and 7 cases of lung cancer. There was no significant difference in gender and age between pulmonary tuberculosis patients and NTM pulmonary disease patients ($P>0.05$), which was comparable.

Comparison of the positive rate of single detection method

The positive rates of FQ-PCR, culture method, colloidal gold method and smear method were

Table 1. FQ-PCR diagnosis results (n)

Clinical diagnosis results	FQ-PCR			Culture method			Colloidal gold method			Smear method		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Tuberculosis	55	5	60	54	6	60	31	29	60	9	52	60
Non-tuberculosis	10	50	60	22	38	60	40	20	60	45	15	60
Total	64	56	120	76	44	120	70	50	120	53	67	120

Table 2. FQ-PCR + culture methods (n)

FQ-PCR + culture method	Clinical diagnosis results		Total
	Tuberculosis	Non-tuberculosis	
Positive	59	6	65
Negative	1	54	55
Total	60	60	120

Table 3. FQ-PCR + colloidal gold diagnosis results (n)

FQ-PCR + colloidal gold method	Clinical diagnosis results		Total
	Tuberculosis	Non-tuberculosis	
Positive	57	12	69
Negative	3	48	51
Total	60	60	120

Table 4. FQ-PCR + smear diagnosis results (n)

FQ-PCR + smear method	Clinical diagnosis results		Total
	Tuberculosis	Non-tuberculosis	
Positive	55	14	69
Negative	5	46	51
Total	60	60	120

Table 5. Diagnostic results of culture method + colloidal gold method (n)

Culture method + colloidal gold method	Clinical diagnosis results		Total
	Tuberculosis	Non-tuberculosis	
Positive	56	15	71
Negative	4	45	49
Total	60	60	120

91.7%, 90.0%, 51.7%, and 16.7%, respectively. The difference among them was statistically significant ($P<0.05$, **Table 1**).

Comparison of the positive rate of two combined detection methods

The positive rates of FQ-PCR + culture method, FQ-PCR + colloidal gold method, FQ-PCR + smear method, culture method + colloidal gold method, culture method + smear method, and colloidal gold method + smear method were 98.3%, 95.0%, 91.7%, 90.0%, 91.7% and 65.0%, respectively. Among them, the positive rate of colloidal gold + smear methods was significantly lower than that of other combined detection methods ($P<0.05$, **Tables 2**-**7**).

Comparison of diagnostic efficiency of single detection method

The sensitivity, specificity and accuracy of FQ-PCR + culture method were significantly higher than those of colloidal gold method + smear method ($P<0.05$), which was suggested that FQ-PCR + culture method showed high diagnostic efficiency in terms of sensitivity, specificity and accuracy in the diagnosis of pulmonary tuberculosis (**Figure 1**).

Comparison of diagnostic efficiency of the two combined methods

The sensitivity, specificity and accuracy of colloidal gold method + smear method were significantly lower than those of FQ-PCR + culture method, FQ-PCR + colloidal gold method, FQ-PCR + smear method, culture method + colloidal gold method, culture method + smear method in the diagnosis of pulmonary tuberculosis ($P<0.05$), of which FQ-PCR + culture method exhibited the best diagnostic efficiency (**Figure 1**).

The incidence of adverse reactions

None of the subjects had serious adverse reactions during the examination. There were 46 cases with a small amount of bloody sputum, 34 cases with mild eye discomfort, and 2 cases with moderate fever after surgery. No special treatment was given and patients recovered spontaneously after 1-3 days (**Table 8**).

Table 6. Diagnosis results of culture method + smear method (n)

Culture method + smear method	Clinical diagnosis results		Total
	Tuberculosis	Non-tuberculosis	
Positive	55	15	70
Negative	5	45	50
Total	60	60	120

Table 7. Diagnostic results of colloidal gold method + smear method (n)

Colloidal gold method + smear method	Clinical diagnosis results		Total
	Tuberculosis	Non-tuberculosis	
Positive	39	26	65
Negative	21	34	55
Total	60	60	120

Discussion

Patients do not develop symptoms immediately after being infected with tuberculosis bacteria. Tuberculosis bacteria can lurk in the human body. The symptoms occur only when the body's immune function is low or the cell-mediated allergic reaction is intensified. Most patients can be cured [10]. At present, bacteriological test is still the main detection method for the clinical diagnosis of tuberculosis, including smear method, acid-fast staining method, and culture method [11, 12]. The smear acid-fast staining method is fast, simple and economical, but its diagnostic sensitivity is low, and the clinical detection rate is only about 35%. At the same time, it is impossible to accurately distinguish *Mycobacterium tuberculosis* and non-tuberculous mycobacteria. The diagnostic specificity is also low [13]. At present, the culture method is still the gold standard for clinical diagnosis of tuberculosis, and its positive detection rate is relatively high. However, due to its longer detection time, it usually takes 4-8 weeks, and rapid training also takes 2 weeks, which cannot help patients diagnose their condition timely, and can easily delay treatment and even lead to disease progression [14-16].

In recent years, the popularization of fiberoptic bronchoscopy has provided great convenience for the clinical diagnosis and treatment of pulmonary tuberculosis. Using fiberoptic bronchoscopy for examination, BALF can be obtainedinthebronchoalveolar,andthedetection of BALF can improve the detection of pathogenic bacteria. The incidence rate and the pollution rate are low. This method can also be used in all patients without sputum [17]. Clinical assays of BALF include smear, culture, PCR, enzymology, *etc*. Among them, PCR detection is significantly more sensitive than smear, culture, and bacterial collection methods [18]. The results of this study showed that the sensitivity, specificity, and accuracy of FQ-PCR and culture methods were significantly higher than those of colloidal gold method and smear method in the diagnosis of pulmonary tuberculosis. Colloidal gold method + smear method showed lower sensitivity, specificity, and accuracy than those of FQ-PCR + culture method, FQ-PCR + colloidal gold method, FQ-PCR + smear method, and culture method + smear method. There were no serious adverse reactions in alveolar lavage and bronchoscopy. It demonstrated that the detection of BALF by FQ-PCR and culture method is helpful to determine the early condition of patients with tuberculosis, and the combination of the two detection methods is conductive to improving the early diagnosis rate of tuberculosis. In recent years, advances in molecular biology technology have brought about FQ-PCR technology. Since the entire process can be done within a closed tube, it is possible to prevent the pollution caused by amplification products. By using specific probes for real-time detection, fast, simple, accurate and specific detection is truly achieved [19-21]. Therefore, FQ-PCR is very sensitive and can detect 10 copies of *Mycobacterium tuberculosis* genomic DNA. Relevant studies have pointed out that [22, 23] the positive rate of FQ-PCR in patients with active pulmonary tuberculosis was as high as 60%, which was higher than that of sputum smear method and modified Roche culture method. Some studies have also indicated that [24] FQ-PCR was used to detect tuberculosis DNA in BALF, and the positive rate was as high as 65%, which was significantly higher than that (6%) of sputum culture and sputum smear methods. Therefore, many scholars believe that the FQ-PCR is a genetic test with the advantages of high specificity and

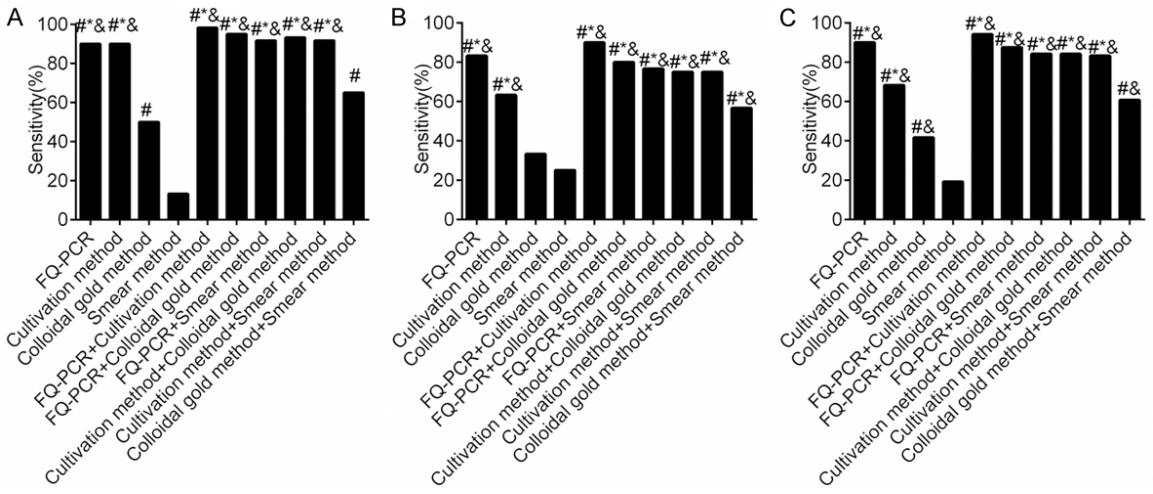

Figure 1. Comparison of the diagnostic efficacy of different detection methods for *Mycobacterium tuberculosis.* Note: Compared with smear method, $^{\#}P<0.05$; compared with colloidal gold method, $^{*}P<0.05$; compared with colloidal gold method + smear method, $^{\&}P<0.05$.

Table 8. Incidence of adverse reactions in patients (n)

Type	Case	Incidence (%)
A small amount of bloody sputum	46	25.56
Mild eye discomfort	34	18.89
Moderate fever	2	1.11

sensitivity, especially in the diagnosis of BALF in atypical tuberculosis patients with dry cough, no sputum and sputum smear-negative, showing high diagnostic value [25]. At present, it is clinically accepted that the detection of BALF is a safe method with fewer adverse reactions than open lung biopsy, and there have been no reports of clinical deaths caused by BALF detection.

In summary, FQ-PCR and culture method on BALF are helpful to determine the condition of patients with pulmonary tuberculosis in the early stage, and the combination of the two detection methods improves the early diagnosis rate of tuberculosis.

Disclosure of conflict of interest

None.

Address correspondence to: Yiqing Luo, Ophthalmology Department, Affiliated Hospital of Qinghai University, No. 29, Tongren Road, Xining 811600, Qinghai Province, China. Tel: +86-18697162716; E-mail: yiqingluo100@163.com

References

[1] Wang Z, Liu Q, Wang X, Wang Y, Zhang J, Zhou W and Yang X. Expressions of microRNA-127-5p in bronchoalveolar lavage fluid of patients with severe pneumonia and its diagnostic value. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 2017; 29: 592-595.

[2] Wang YX, Fang F, Guo YF, Li YM, Sun TY, Zhang M, Chen J and Fang BM. Analysis of 12 cases of exogenous lipoid pneumonia confirmed by pathology. Zhonghua Jie He He Hu Xi Za Zhi 2017; 40: 445-449.

[3] Heldt S, Prattes J, Eigl S, Spiess B, Flick H, Rabensteiner J, Johnson G, Prüller F, Wölfler A, Niedrist T, Boch T, Neumeister P, Strohmaier H, Krause R, Buchheidt D and Hoenigl M. Diagnosis of invasive aspergillosis in hematological malignancy patients: performance of cytokines, Asp LFD, and Aspergillus PCR in same day blood and bronchoalveolar lavage samples. J Infect 2018; 77: 235-241.

[4] Yan L, Zhang Q and Xiao H. Clinical diagnostic value of simultaneous amplification and testing for the diagnosis of sputum-scarce pulmonary tuberculosis. BMC Infect Dis 2017; 17: 545.

[5] Ullah I, Javaid A, Masud H, Ali M, Basit A, Ahmad W, Younis F, Yasmin R, Khan A, Jabbar A, Husain M and Butt ZA. Rapid detection of mycobacterium tuberculosis and rifampicin resistance in extrapulmonary tuberculosis and sputum smear-negative pulmonary suspects using Xpert MTB/RIF. J Med Microbiol 2017; 66: 412-418.

[6] Zhou W, Li H, Zhang Y, Huang M, He Q, Li P, Zhang F, Shi Y and Su X. Diagnostic value of galactomannan antigen test in serum and

bronchoalveolar lavage fluid samples from patients with nonneutropenic invasive pulmonary aspergillosis. J Clin Microbiol 2017; 55: 2153-2161.
[7] Xiao W, Gong DY, Mao B, Du XM, Cai LL, Wang MY and Fu JJ. Sputum signatures for invasive pulmonary aspergillosis in patients with underlying respiratory diseases (SPARED): study protocol for a prospective diagnostic trial. BMC Infect Dis 2018; 18: 271.
[8] Urabe N, Sakamoto S, Sano G, Suzuki J, Hebisawa A, Nakamura Y, Koyama K, Ishii Y, Tateda K and Homma S. Usefulness of two aspergillus pcr assays and aspergillus galactomannan and β-d-glucan testing of bronchoalveolar lavage fluid for diagnosis of chronic pulmonary aspergillosis. J Clin Microbiol 2017; 55: 1738-1746.
[9] Zhang P. Guidelines for diagnosis and treatment of tuberculosis. Zhonghua Jie He He Hu Xi Za Zhi 2001; 24: 70-74.
[10] Lu D, Yao X, Abulimiti A, Cai L, Zhou L, Hong J and Li N. Profiling of lung microbiota in the patients with obstructive sleep apnea. Medicine (Baltimore) 2018; 97: e11175.
[11] Suzuki A, Taniguchi H, Kondoh Y, Ando M, Watanabe N, Kimura T, Kataoka K, Yokoyama T, Sakamoto K and Hasegawa Y. Soluble thrombomodulin in bronchoalveolar lavage fluid is an independent predictor of severe drug-induced lung injury. Respirology 2017; 22: 744-749.
[12] Maruyama K, Inaba T, Tamada T and Nakazawa T. Vitreous lavage fluid and bronchoalveolar lavage fluid have equal diagnostic value in sarcoidosis. Medicine (Baltimore) 2016; 95: e5531.
[13] Desoubeaux G, Cabanne É, Franck-Martel C, Gombert M, Gyan E, Lissandre S, Renaud M, Monjanel H, Dartigeas C, Bailly É, Van Langendonck N and Chandenier J. Pulmonary toxoplasmosis in immunocompromised patients with interstitial pneumonia: a single-centre prospective study assessing PCR-based diagnosis. J Clin Pathol 2016; 69: 726-730.
[14] Wang Y, Li D, Qiao L and Zhao F. Infant central nervous system aspergillosis with first-episode of intracranial hemorrhage: a case report. Medicine (Baltimore) 2017; 96: e8893.
[15] Vernikos P, Kampolis CF, Konstantopoulos K, Armaganidis A and Karakitsos P. The role of bronchoscopic findings and bronchoalveolar lavage fluid cytology in early diagnosis of ventilator-associated pneumonia. Respir Care 2016; 61: 658-667.
[16] Song JI, Kim YK, Hwang JH and Yang HJ. Early diagnosis based on clinical history and BALF for successful management of smoking-induced acute eosinophilic pneumonia without unnecessary antibiotic usage: a case report. J Asthma 2016; 53: 452-455.
[17] Sugui JA, Rose SR, Nardone G, Swamydas M, Lee CR, Kwon-Chung KJ and Lionakis MS. Host immune status-specific production of gliotoxin and bis-methyl-gliotoxin during invasive aspergillosis in mice. Sci Rep 2017; 7: 10977.
[18] Fortún J, Martín-Dávila P, Gomez Garcia de la Pedrosa E, Silva JT, Garcia-Rodríguez J, Benito D, Venanzi E, Castaño F, Fernández-Ruiz M, Lazaro F, García-Luján R, Quiles I, Cabanillas JJ, Moreno S and Aguado JM. Galactomannan in bronchoalveolar lavage fluid for diagnosis of invasive aspergillosis in non-hematological patients. J Infect 2016; 72: 738-744.
[19] Wang J, Liu I, Yue Y, Hu Y, Fan D, Li H, Zhang Y, Xia Q and Zhu M. Combined detection of alveolar lavage fluid and real-time detection technology of RNA constant temperature amplification and fluorescent quantitative PCR for rapid diagnosis of sputum smear-negative pulmonary tuberculosis. Chinese J Nosocomiol 2017; 300-304, 320.
[20] Wayengera M, Mwebaza I, Welishe J, Bayiyana A, Kateete DP, Wampande E, Kirimunda S, Kigozi E, Katabazi F, Musubika C, Kyobe S, Babirye P, Asiimwe B and Joloba ML. Immunodiagnosis of mycobacterium tuberculosis in sputum, and reduction of timelines for its positive cultures to within 3 h by pathogen-specific thymidylate kinase expression assays. BMC Res Notes 2017; 10: 368.
[21] Zhu C, Zhao YB, Kong LF, Li ZH and Kang J. The expression and clinical role of KL-6 in serum and BALF of patients with different diffuse interstitial lung diseases. Zhonghua Jie He He Hu Xi Za Zhi 2016; 39: 93-97.
[22] Singhal R, Reynolds PR, Marola JL, Epperson LE, Arora J, Sarin R, Myneedu VP, Strong M and Salfinger M. Sequence analysis of fluoroquinolone resistance-associated genes gyrA and gyrB in clinical mycobacterium tuberculosis isolates from patients suspected of having multidrug-resistant tuberculosis in New Delhi, India. J Clin Microbiol 2016; 54: 2298-2305.
[23] Deng J, Wu SY, Liu Y, Ma Y, Xie Y, Chen ZX, Dai ZQ, Deng JL and Kang M. Galactomannan tests of bronchoalveolar lavage fluid for diagnosing invasive pulmonary aspergillosis. Sichuan Da Xue Xue Bao Yi Xue Ban 2018; 49: 124-128.
[24] Jarikre AT, Emikpe OB, Ohore GO, Akinremi AT and Akpavie OS. Bronchoalveolar lavage fluid cellular and haematological changes in different types of caprine pneumonia. Niger J Physiol Sci 2016; 31: 31-36.
[25] Takahashi Y, Saito A, Chiba H, Kuronuma K, Ikeda K, Kobayashi T, Ariki S, Takahashi M, Sasaki Y and Takahashi H. Impaired diversity of the lung microbiome predicts progression of idiopathic pulmonary fibrosis. Respir Res 2018; 19: 34.