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Original Article

Pathogenic microorganism detection in AIDS patients using bronchoalveolar lavage fluid

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Abstract: This study aimed to investigate the use of fiberoptic bronchoscopy and bronchoalveolar lavage in the diagnosis of pulmonary pathogenic microorganism infection in AIDS patients. We retrospectively analyzed the clinical data, fiberoptic bronchoscopy and bronchoalveolar lavage fluid laboratory examinations of 209 AIDS patients with pulmonary infection. Among 209 patients, we found 42 cases of mycobacterial infection, 3 cases of bacterial infection, 58 cases of pneumocystis carinii pneumonia (PCP), 27 cases that were fungal positive, 99 cases of CMV, and 103 cases positive for GM test of which 83 cases were considered *Aspergillus* positive. BALF pathogen distribution was related to CD4+ T lymphocyte count. The primary pathogens of pulmonary infection in AIDS patients were cytomegalovirus, *Mycobacterium tuberculosis*, fungi, and *Pneumocystis carinii*. Fiberoptic bronchoscopy and bronchoalveolar lavage are important in the diagnosis of pathogenic microorganisms in lung infections of AIDS patients.

Keywords: AIDS, BALF, pulmonary infection, microorganism

Introduction

Pulmonary infection is one of the most common complications of AIDS patients, with a high mortality. Early diagnosis and targeted treatment are key to improving prognosis [1]. The pathogens causing pulmonary infection include fungi, viruses, or *Mycobacterium tuberculosis* [2]. Due to the complexity of the pathogens and the lack of specific clinical and chest radiography findings, the diagnosis of lung infections in AIDS patients is still challenging.

Pulmonary infection in AIDS patients tends to be severe, and often combined with respiratory failure which may be life-threatening [3, 4]. Clinical diagnosis of pulmonary infections generally includes microscopic examination of smears or cultures of respiratory specimens, galactomannan (GM) test, cryptococcal latex agglutination test [5, 6], PCR-based genetic diagnosis methods [7], and chest imaging examinations [8, 9]. Previous studies have shown that combined use of diagnostic methods might improve the diagnosis of pulmonary infection [7, 10, 11]. Respiratory specimens for clinical diagnosis mainly include sputum drainage, bronchoscopy, bronchoalveolar lavage fluid (BALF), and lung biopsy.

In this study, we studied 209 cases by bronchoscopy and BALF in terms of the pathogenic microorganism composition, and we discuss the value of bronchoalveolar lavage (BAL) in the diagnosis of pulmonary infection in AIDS patients.

Material and methods

Patients and fiberoptic bronchoscopy

209 AIDS patients with pulmonary infection were recruited for bronchoscopy in the Xixi Hospital of Hangzhou China, from May 2016 to June 2019. All AIDS patients met the diagnostic criteria of AIDS treatment guidelines. Among 209 patients, 193 were males and 16 were females. The age ranged from 18 to 81 years, with an average age of 37.9 years old. Patients were subjected to lymphocyte subset analysis

Table 1. BALF pathogens and infection rate in 209 AIDS patients

Pathogen	Number of infections	Infection rate
Cytomegalovirus	99	47.4%
Pneumocystis carinii	58	27.8%
Cryptococcus neoformans	13	6.2%
Talaromyces marneffei	14	6.7%
Mycobacterium tuberculosis	34	16.3%
Mycobacterium avium	8	3.8%
Aspergillus	83	39.7%
Pseudomonas aeruginosa	3	1.4%

by flow cytometry to determine CD4+ T lymphocyte counts. One week after hospitalization BAL was performed after bronchoscopy. This study was approved by the Ethics Committee of Xixi Hospital of Hangzhou. The participant consent was written and was performed in accordance with the ethical standards of the Declaration of Helsinki of 1964.

BALF cytology test

Slides of the BALF samples were prepared for differential cell counts by centrifugation of undiluted samples and subsequent smear of the cell pellet [12]. Acid-fast staining, fluorescent staining and Mycobacterium tuberculosis culture were performed according to Fukunaga et al.'s method [13]. The Cytomegalovirus (CMV) DNA load detection described by Okahara et al. was performed [14]. Hexamine silver staining techniques and PCR were performed to diagnose pneumocystis carinii pneumonia (PCP) in BALF [15, 16]. GM detection was performed to detect aspergillus infection [5, 17].

qPCR

The DNA extracted from BALF was performed with the QIAamp DNA kit (Qiagen, France) according to the manufacturer's instructions. Before extraction, all samples were centrifuged at 3,000 rpm for 5 min. The qPCR was performed according to Noguchi et al.'s method [18]. The primers described by Wakefield et al. [19] for the amplification of a part of the mitochondrial gene encoding for the large subunit of rRNA were used: pAZ102E (5'-GATGGCTGTTTCCAAGCCCA-3') and pAZ102H (5'-GTGTACGTTGCAAAGTACTC-3').

Statistical analysis

Statistical analyses were performed with SPSS 11.5 software. Measurement data are presented as mean ± standard deviation ($x \pm s$). For the statistical analyses, the t-test and χ^2 test were used, and a value of $P<0.05$ was considered significant.

Results

BALF pathogen distribution

Among the 209 AIDS patients who underwent BAL, 189 patients were positive for BALF pathogenic assays including smear, culture, and PCR, with the positive rate at 90.4%. 99 cases were positive in CMV DNA load detection (>500 cp/ml) (49 cases of CMV DNA load $<10^4$ cp/ml; 28 cases of 10^4~10^5 cp/ml; 22 cases $>10^5$ cp/ml), the positive rate of 47.4%. Hexamine silver staining and PCR confirmed 58 cases were *Pneumocystis carinii* positive. Fungal smear and culture showed 27 cases positive (13 cases of *Cryptococcus neoformans*, 14 *Talaromyces marneffei*). Acid-fast staining smear, fluorescent staining, and culture revealed 42 *Mycobacterium* cases including 34 cases of *Mycobacterium tuberculosis* and 8 cases of *Mycobacterium avium*. GM results showed 103 cases were positive (cut-off value >0.5, 83 cases of *Aspergillus*, 14 cases of *Talaromyces marneffei*, 6 cases of *Cryptococcus neoformans*). 3 cases of bacterial culture were positive (*Pseudomonas aeruginosa*). Of all the BALF pathogenic positive patients, 47.1% were single pathogen infected (89 cases), 42.3% were double infected (80 cases), and 10.6% were triple infected (20 cases) (**Tables 1** and **2**). In addition, 10.0% (21 cases) of 209 patients were CMV DNA positive in serum and 38.8% (81 cases) in urine.

Relationship between CD4+ T lymphocyte counts and BALF pathogen distribution

Among the 189 BALF pathogenic positive patients, 114 patients' (60.3%) CD4+ T lymphocyte counts were <50/μl, 53 patients' (28.0%) CD4+ T lymphocyte counts were 50-200/μl, and 22 patients' (11.6%) CD4+ T lymphocyte counts were >200/μl. The difference was statistically significant ($\chi^2=6.645$, $P<0.05$) (**Table 3**).

Table 2. Distribution and composition ratio of BALF pathogens in 189 AIDS patients

Pathogen	Number of cases	Composition ratio (%)
Single pathogen	89	47.09
Cytomegalovirus	27	14.29
Pneumocystis carinii	28	14.81
Mycobacterium tuberculosis	10	5.29
Aspergillus	17	8.99
Cryptococcus neoformans	3	1.59
Talaromyces marneffei	4	2.12
Double infection	80	42.33
Cytomegalovirus/Aspergillus	24	12.70
Cytomegalovirus/Cryptococcus neoformans	8	4.23
Cytomegalovirus/Pneumocystis carinii	4	2.12
Cytomegalovirus/Mycobacterium avium	8	4.23
Cytomegalovirus/Talaromyces marneffei	8	4.23
Aspergillus /Pneumocystis carinii	16	8.47
Aspergillus/Mycobacterium tuberculosis	8	4.23
Mycobacterium tuberculosis/Cryptococcus neoformans	2	1.06
Mycobacterium tuberculosis/Talaromyces marneffei	2	1.06
Triple infection	20	10.58
Cytomegalovirus/Mycobacterium tuberculosis/Aspergillus	10	5.29
Cytomegalovirus/Pneumocystis carinii/Aspergillus	8	4.23
Cytomegalovirus/Mycobacterium tuberculosis/Pneumocystis carinii	2	1.06
Total	189	100.00

Table 3. Relationship between CD4+ T lymphocyte count and distribution of BALF pathogens

Pathogen	N	CD4+ T lymphocyte count		
		<50/μl	50~200/μl	>200/μl
Single pathogen	89	49	24	16
Compound infection	100	65	29	6
Pathogen negative	20	9	4	7
Total	209	123	57	29

Discussion

In our study, the positive rate of pathogenic diagnosis of lung infection was 90.4% (189/209). The pathogens were mainly CMV, *Pneumocystis carinii*, *Aspergillus*, *Cryptococcus*, *Talaromyces marneffei*, and *Mycobacterium*. The higher pulmonary infection rates are related to immunodeficiency. Interestingly, only 3 cases were positive for bacterial culture, considering these patients accepted extensive spectrum antibiotics before and after admission. Although the patients have low immunity and lung infection, fiberoptic bronchoscopy and bronchoalveolar lavage were well tolerated, and no obvious complications were observed after examination. This indicated that fiberoptic bronchoscopy and bronchoalveolar lavage is a valuable technique showing safety in AIDS patients.

CMV is a common opportunistic pathogen of infection, but the detection sensitivity is poor. The seroprevalence of CMV in different populations and regions is 40-100% [20]. The lungs were the main latent sites of CMV, mostly in patients with CD4+ T <50/ul. Our results showed that the positive rate of CMV DNA was 47.4% (99/209) in BALF, 10.0% in serum, and 38.8% in urine, indicating that CMV quantitative PCR is more sensitive in BALF. However, whether CMV DNA positive in BALF can be diagnosed as cytomegalovirus pneumonia (CMVP) needs further confirmation. One study reported that CMV activation was an indicator of immunosuppressive state and disease severity and did not require treatment [21]. Xue *et al.* found that CMV load >1.75 × 10^4 cp/ml in rheumatism BALF showed diagnostic value in CMVP [22]. CMVP often combines with *Mycobacteria*, *Pneumocystis carinii*, and fungi, which can cause fever, cough, dyspnea, and interstitial pneumonia. Besides, it was found

that a higher CMV DNA load is significantly associated with mortality [23]. The combined infection of CMV in this group was 34.4% (72/209), so the detection of CMV DNA by BALF can be done in early stage for early anti-virus treatment.

Pneumocystis carinii is a common opportunistic infection of AIDS, and nucleic acid detection is generally used due to the lack of reliable *in vitro* culture methods. The detection of *Pneumocystis* DNA in the respiratory tract without clinical manifestations is called *Pneumocystis* colonization [24]. The literature reports showed that 20.0% of adult oral rinses, 44.0% of non-HIV patients were positive by BALF, and 46.0% of HIV-infected patients were *Pneumocystis* DNA positive [25-27]. The infection rate of BALF *Pneumocystis* in this study was 27.8% (58/209). In AIDS patients, *Pneumocystis* combined with CMV co-infection is common at 28~69% [28]. Our results showed CMV combined with *Pneumocystis* was 6.7% (14/209), much lower than in other studies. This might be due to the active anti-*Pneumocystis* treatment after patients were admitted to the hospital.

GM is a polysaccharide component located on the cell wall of *Aspergillus* cells, which is released into blood and body fluids during the invasion of human tissues. A retrospective study found that the detection of BALF-GM is helpful in the diagnosis of invasive pulmonary aspergillosis (IPA) [29]. The EORTC/MSGIPA guidelines consider GM >0.5 to be a criterion for diagnosis of IPA. Gupta *et al*. found that a cut-off value >1 detected by BALF-GM will increase the specificity of diagnostic IPA [30]. In our study, 103 cases detected GM >0.5 (49.3%), including 83 cases of *Aspergillus*, supporting BALF-GM as a sensitive approach.

AIDS patients have severely impaired immune function, repeated hospitalization, prolonged treatment, and administration of various antibacterial drugs, resulting in continuous changes in pathogens. Multiple infections are common in patients with CD4+ T cells <200/μl, especially patients with CD4+ T cells <50/μl. In our study, 180 patients exhibited CD4+ T cells <200/μl, 100 patients developed multiple infections, 65 of which had CD4+ T cells <50/μl, suggesting that multiple infections should be noted based on T cell counting for early treatment. According to the clinical manifestations, a broad spectrum of anti-bacterial, anti-fungal, and anti-CMV treatment options can be selected for immunodeficiency patients with pulmonary infection based on bronchoscopy.

Some limitations of the study need to be pointed out. Many pathogens have not been tested in our hospital due to limited approaches of microorganism detection. The pathogens associated with a lung infection in AIDS patients also include respiratory syncytial virus, influenza virus, herpes virus, and mycoplasma. In addition, we were unable to identify pathogens in 20 patients (9.6%). Therefore, with the help of pathogenic microorganism detection by fiberoptic bronchoscopy and bronchoalveolar lavage, combined with clinical manifestations and imaging features, empirical treatment can be performed to improve the prognosis of patients as soon as possible effectively. However, more in-depth and multi-center, large-sample studies are needed of the long-term benefit of fiberoptic bronchoscopy and bronchoalveolar lavage examinations.

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Disclosure of conflict of interest

None.

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