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Utility of flexible bronchoscopy with polymerase chain reaction in the diagnosis and management of pulmonary infiltrates in allogeneic HSCT patients

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Abstract

Objectives: Pulmonary infiltrates in allogeneic hematopoietic stem cell transplant (allo-HSCT) patients are potentially life-threatening and require early diagnosis and treatment. We aimed to retrospectively explore the clinical efficacy of polymerase chain reaction (PCR) in conjunction with flexible bronchoscopy (FB) in allo-HSCT patients with pulmonary infiltrates.

Patients and methods: We retrospectively reviewed all patients undergoing FB after allo-HSCT at the Peking University Institute of Hematology from January 2013 to December 2016. We used PCR to detect various viruses in FB specimens, particularly for 27 viruses.

Results: One hundred forty-nine diagnostic FBs were performed in 130 patients. The overall diagnostic yield was 58%. Eighty-nine percent of the patients with a positive FB result were diagnosed with a pulmonary infection. Viruses were the most common infectious diagnosis (70%), followed by fungi (48%), bacteria (38%), and *Pneumocystis jirovecii* (12%). Multivariate analyses showed that a chest computed tomography (CT) finding of diffuse pulmonary infiltrates (P = .012) and positive results in assisted microbiological and serological analyses (P = .000) predicted a positive FB result. FB results prompted a treatment modification in 61% of cases.

Conclusions: FB in conjunction with PCR is efficient in the rapid diagnosis and management of pulmonary infiltrates in allo-HSCT patients.

KEYWORDS
flexible bronchoscopy, hematopoietic stem cell transplantation, polymerase chain reaction, pulmonary infiltrate

1 | INTRODUCTION

Both infectious and noninfectious pulmonary complications remain the major causes of morbidity and mortality within the allogeneic hematopoietic stem cell transplant (allo-HSCT) population.[1] The rapid diagnosis and treatment of these complications improves survival.[2] Diagnoses may be difficult because the clinical symptoms or radiological signs revealed in these cases are nonspecific. Furthermore, the complication rate of lung biopsies limits its use even if the highest yield.[3,4] Flexible bronchoscopy (FB) remains the traditional initial investigative method in allo-HSCT patients with pulmonary infiltrates.[3-13]

Previous studies have examined the use of FB as the main diagnostic method in the HSCT population and have reported yields of 23%-65%.[4-13] However, most of these studies have various limitations,

such as small sample sizes and the inclusion of other nontransplant or autotransplant populations. Additionally, the methods for detecting viruses in FB specimens in most of the previous studies have included viral cultures,[5,10] viral isolation,[7] the identification of inclusion bodies,[8] or viral antigens using direct immunofluorescence or immunohistochemical analyses.[6,7,10] These methods have low positive rates and require long detection times. Prompt and sensitive tests are needed to identify specific pathogens, thereby allowing the early administration of appropriate therapies. Polymerase chain reaction (PCR) is a useful alternative diagnostic tool. Few studies have reported the use of PCR to detect various viruses in FB specimens.[4,12] Oren et al[4] reported the use of PCR for seven viruses in diagnostic FB, while Brownback et al[12] reported the use of PCR for only two viruses in FB.

The aim of our study was to retrospectively explore the clinical efficacy of PCR, particularly for 27 viruses, in conjunction with FB in allo-HSCT patients with pulmonary infiltrates.

2 | PATIENTS AND METHODS

2.1 | Patients

All consecutive patients with pulmonary infiltrates after allo-HSCT who underwent diagnostic FB between January 2013 and December 2016 were included in this study. In patients who underwent more than one FB, each examination was considered independently. All patients in this study had a high-resolution computed tomography (CT) scan of the chest performed prior to the FB. The pulmonary infiltrates were analyzed based on the final radiological report, which indicated the absence of infiltrates or the presence of focal infiltrates (which affected one or two lobes) or diffuse infiltrates (which affected more than two lobes). Treatment modification was defined as the addition, discontinuation, or change in antimicrobial treatment or the addition of systemic corticosteroids due to the bronchoscopic results within 3 days after FB. Neutropenia was defined as an absolute neutrophil count $<1 \times 10^9$/L. The progression of infiltrates resulting in respiratory failure and death within 30 days following FB was recorded as pulmonary-associated mortality. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committee of Peking University People's Hospital.

2.2 | Transplant procedure

All patients received a myeloablative conditioning regimen (MAC) without in vitro T-cell depletion. The conditioning therapy was modified BU/CY plus ATG for haplo-HSCT and unrelated donor (URD) HSCT[14,15] and was modified BU/CY for the identical sibling donor (ISD) group. Patients in the haplo-HSCT and ISD groups received fresh granulocyte colony-stimulating factor (G-CSF)-mobilized and unmanipulated G-CSF-primed bone marrow cells in addition to G-CSF-primed peripheral blood stem cells (PBSCs). Patients in the URD HSCT group received fresh G-CSF-mobilized and unmanipulated PBSCs. All patients received cyclosporine, mycophenolate mofetil, and short-term methotrexate for GVHD prophylaxis.[14,15]

2.3 | Flexible bronchoscopy

Bronchoscopies were performed by a respiratory physician using a flexible fiber-optic bronchoscope (Olympus). Procedures were performed through the nasal or oral cavity following local anesthesia (lidocaine 2%) and were supported with cardiopulmonary monitoring (continuous assessment of pulse rate, blood pressure, and oxygen saturation). All FB samples were obtained from areas of lung infiltration. If multiple areas were present, samples were obtained from the area where the infiltration was most severe.

Various flexible bronchoalveolar procedures, including bronchoalveolar lavage (BAL), protected sample brushing (PSB), and transbronchial biopsy (TBB), have been used to establish a diagnosis. The decision to request PSB or TBB was made by the respiratory physician responsible for the FB. Among patients who underwent multiple FBs, each procedure was recorded and considered independently.

2.4 | Microbiologic detection

Blood and FB samples were routinely subjected to the following tests: (i) Gram stain, fungal stain, Grocott-Gomori methenamine-silver stain, and acid-fast bacilli staining; (ii) cytology examination; (iii) bacterial and fungal cultures; (iv) galactomannan (GM); and (v) real-time PCR and reverse transcription PCR for the detection of atypical bacteria (eg, Legionella species, Mycoplasma pneumonia, and atypical mycobacteria), *Pneumocystis jirovecii*, and the following 27 viruses: herpesviruses (HSV types 1 and 2, cytomegalovirus [CMV], Epstein-Barr virus [EBV], varicella zoster virus, and human herpesvirus-6), respiratory viruses (influenza types A [including A-H1N1] and B, parainfluenza virus, coxsackievirus A16, respiratory syncytial virus [RSV-A/B], human bocavirus, human metapneumovirus, human coronaviruses [CoVs:OC43, 229E, NL63, and HKU1], human rhinoviruses, and adenovirus), polyomaviruses (BK virus and JC virus), parvovirus B19, and enteroviruses (norovirus, rotavirus, enterovirus general type, and enterovirus 71).[16] CMV and EBV test results showing >1000 and >500 viral copies/mL in the FB samples, respectively, were defined as positive. PCR for other viruses was qualitative. Assistant microbiological and serological analyses included the following: peripheral blood cultures, sputum cultures, serum GM, serum (1,3)-β-D-glucan assays, and PCR for viruses in blood.

2.5 | Diagnostic criteria

Bacterial pneumonia was defined as the presence of a positive FB culture and/or PCR when strong clinical suspicion of lower respiratory tract infection (eg, the presence of fever, cough, purulent sputum, pleurisy, or leukocytosis).

Fungal pneumonia was defined as a positive FB culture for any mold or rare yeast. Invasive pulmonary aspergillosis (IPA) was defined according to the revised EORTC/MSG criteria,[17] accepting positive GM in blood or BAL samples. BAL GM test was considered positive with a threshold value of 0.5.[6] A positive culture of *Aspergillus* species from FB sample was considered sufficient to diagnose pulmonary

aspergillosis. However, FB cultures positive for Candida were not considered sufficient to diagnose Candida pneumonia unless the strongly clinical grounds, suspicious radiological appearances, and a good clinical response to empirical antifungal agents.

Viral pneumonia was defined as the identification of (i) presence of clinical symptoms of pneumonia; (ii) a positive FB virus PCR for any respiratory virus; and (iii) typical imaging CT features. For other viruses, additional requirements were (i) ± a positive PCR in blood meanwhile; (ii) excluding other infectious pneumonia.

Pneumocystis jirovecii pneumonia (PJP) was defined in the presence of *P. jirovecii* bodies visualized with silver/methenamine stains and/or *P. jirovecii* DNA detected by PCR in BAL fluid obtained from patients with established predisposing factors and typical clinical and imaging CT features.

A polymicrobial pulmonary infection was defined as the identification of two or more infectious agents based on the above criteria. The diagnostic yield of FB was considered to be the proportion of cases with a specific diagnosis.

Diffuse alveolar hemorrhage (DAH) was defined by BAL samples with increasingly bloody return (three separate samples were required) or by the presence of 20% or more hemosiderin-laden macrophages.[18]

2.6 | Statistical analysis

Data were censored at the time of death or the last available follow-up. Continuous variables were compared using the Mann-Whitney U test; categorical variables were compared using the chi-squared test and Fisher's exact test. The Kaplan-Meier method was used to estimate the probability of OS. Univariate analysis was performed using binary logistic regression, calculating the odds ratios (OR) with 95% confidence intervals (CI) and *P*-values. Multivariable stepwise logistic regression analysis was used to assess the relationship between patient characteristics and positive FB result. $P < .05$ was considered statistically significant. We attempted to enter all variables with a *P*-value<.1 in the univariate analysis into the multivariate analysis using the logistic regression. All *P*-values were obtained using 2-sided hypothesis tests. The data analyses were conducted primarily using the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Clinical features

In total, 149 diagnostic FBs were performed in 130 patients after allo-HSCT from January 2013 to December 2016. The following techniques and their frequency were applied during the FB: BAL, 147; PSB, 4; and TBB, 8. BAL was performed twice in nine patients and three times in four patients. The basic characteristics of the patients are shown in Table 1. The median age was 36 years (range 11-64 years). The most common hematological diagnosis was acute myeloid leukemia (AML) (47%, n = 61). The median time between HSCT and FB was 176 days (range 17-1480 days). The median time from CT until FB

TABLE 1 Clinical characteristics of the patients who underwent FB

Characteristics	N = 130
Median age at HSCT, years (range)	36 (11-64)
Age≥35 years, n (%)	70 (54)
Age<35 years, n (%)	60 (46)
Gender, n (%)	
Male	86 (66)
Female	44 (34)
Underlying disease, n (%)	
AML	61 (47)
ALL	35 (27)
MDS	22 (17)
SAA	4 (3)
CML	4 (3)
NHL	2 (1.5)
CMML	2 (1.5)
Donor type	
ISD	27 (21)
HID	97 (74)
URD	6 (5)
Conditioning regimen, n (%)	
TBI based	10 (9)
Non-TBI based	120 (91)
Time from allo-HSCT to FB, days (range)	176 (17-1480)
Time from CT until FB, days (range)	8 (0-86)
Neutrophil granulocyte at time of FB,$\times 10^9$/L (range)	3.15 (0-28.3)
Neutropenia at time of FB, n (%)	
Yes	29 (22)
No	101 (78)
PLT at time of FB,$\times 10^9$/L (range)	69 (10-391)
PLT at time of FB <50 × 10^9/L, n (%)	
Yes	40 (31)
No	90 (69)
Chest CT finding, n (%)	
Diffuse infiltrates	119 (92)
Focal infiltrates	11 (8)
Median time of antimicrobial therapy before FB, days (range)	15 (0-93)
Treatment before FB, n (%)	
antibiotic drugs	127 (98)
antifungal drugs	123 (95)
antiviral drugs	85 (65)
anti-*Pneumocystis jirovecii*	79 (61)

HSCT, hematopoietic stem cell transplantation; AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; MDS, myelodysplastic syndrome; SAA, severe aplastic anemia; CML, chronic myelogenous leukemia; NHL, non-Hodgkin lymphoma; CMML, chronic myelomonocytic leukemia; ISD, identical sibling donor; HID, haploidentical donor; URD, unrelated donor; TBI, total body irradiation; FB, flexible bronchoscopy; PLT, platelets; CT, computed tomography.

TABLE 2 Distribution of the etiological agents of pulmonary infections in allogeneic HSCT patients

Pathogens identified from FB samples	Number
Total number of pathogens	142
Viruses, total	72
Herpesviruses	27
CMV	20
EBV	5
Herpesviruses (HSV types 1 and 2)	1
Human herpesvirus-6	1
Respiratory viruses	38
Respiratory syncytial virus	19
Parainfluenza virus	9
Human coronaviruses	2
Human rhinoviruses	2
Influenza A virus (H1N1)	3
Influenza B virus	2
Human bocavirus	1
Enterovirus	5
Coxsackie virus	4
Enterovirus 71	1
Polyomavirus	2
BK virus	1
JC virus	1
Fungi, total	28
Molds	24
Aspergillus spp	23
Zygomycetes	1
Yeasts	4
Candida	4
Pneumocystis jirovecii	9
Bacteria, total	33
Enterobacteriaceae	8
Pseudomonas aeruginosa	7
Stenotrophomonas maltophilia	1
Acinetobacter	5
Legionella pneumophila	1
Burkholderia cepacia	1
Staphylococcus	3
Tuberculosis	7

CMV, cytomegalovirus; EBV, Epstein-Barr virus.

was 8 days (range 0-86 days). Ninety-seven patients (74%) had undergone haplo-HSCT.

Only three asymptomatic patients were referred for FB when abnormalities were discovered incidentally upon CT, while other patients (n = 127) had a fever or chest symptoms, such as cough, sputum production, dyspnea, and pleuritis. At the time of bronchoscopy, 22% of the patients (n = 29) were neutropenic, while seven patients (5%) were severe neutropenia (ANC <0.5 × 10^9/L). Thrombocytopenia was common (median platelet count of 69 × 10^9/L, range 10–391 × 10^9/L). One hundred nineteen (92%) CT showed diffuse bilateral infiltrates.

All patients received empirical antimicrobial therapy prior to FB. The median duration of antimicrobial therapy before FB was 15 days (range 0-93 days). These therapies included broad-spectrum antibiotics (n = 127, 98%), antifungals (n = 123, 95%), antivirals (n = 85, 65%), and anti-*P. jirovecii* agents (n = 79, 61%). Thirty patients received mechanical ventilation in an intensive care unit before FB.

3.2 | FB results

Table 2 shows details of FB results. The overall diagnostic yield of FB was 58% (87/149), including 63% for BAL (82/147), 25% for PSB (1/4), and 88% for TBB (7/8). FB revealed 10 cases of noninfectious etiology (DAH), and 77 (77/149, 52%) cases were diagnosed with a pulmonary infection. This result implied that 89% (77/87) of positive FB were diagnosed with pulmonary infections.

The most common infectious diagnosis was viral pneumonia (54/77,70%). All viral pneumonias were diagnosed by PCR, and viruses were detected 72 times. CMV was the most commonly detected virus (n = 20). The second common virus was RSV (n = 19). The third common virus was parainfluenza virus (n = 9). Fungal pneumonia was diagnosed in 37/77 cases (48%), with *Aspergillus* spp being the fungal pathogen in 23 (23/37, 62%). Bacterial pneumonia was diagnosed in 29/77 (38%) cases: nonfermentative bacteria in 12 (41%) cases and tubercle bacillus in seven (29%) cases. One case of Legionella pneumonia and one case of tubercle bacillus (TB) were diagnosed by PCR. PJP was diagnosed in 9/77 (12%) cases. The diagnosis of PJP was confirmed by PCR in 8/9 (89%) cases, and in the one remaining case, the organism was visualized by direct staining.

A polymicrobial etiology was established in 47% of patients (36/77): two microorganisms in 19 patients; three microorganisms in 12 patients; and four microorganisms in five patients. *Aspergillus* spp was the most common co-pathogen in polymicrobial pulmonary infections group (15/36, 42%). Pathogens were also identified in assisted microbiological and serological analyses in 76 patients: 44 pathogens were in accord with FB results, while 32 were different from FB results. There were no positive blood cultures.

3.3 | Prediction of a positive FB result

The univariate analysis demonstrated that non-ISD (P = .012), CT findings of diffuse infiltrates (P = .003), PLT<50 × 10^9/L (P = .072), and positive assisted microbiological and serological analyses (P = .000) were statistically or marginally associated with a higher likelihood of obtaining a positive FB result (Table 3). In the multivariate analysis, only CT findings of diffuse infiltrates (P = .004) and positive results in assisted microbiological and serological analyses (P = .000) were significantly associated with a higher likelihood of obtaining a positive FB result. The diagnostic yield was significantly improved in patients with diffuse infiltrates compared with patients who had focal infiltrates (98% vs 80%, P = .003). Positive results in assisted microbiological and

TABLE 3 Multivariate analyses of factors predicting a positive FB result

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	***P*-Value**	**HR**	**95% CI**	***P*-Value**
Donor type						
ISD	1		.009	1		.063
Non-ISD	3.19	1.333-7.63		2.57	0.949-6.962	
PLT<50 × 10^9/L at time of FB						
No	1		.072	1		.299
Yes	2.192	0.933-5.512		1.682	0.63-4.49	
Chest CT finding						
Focal infiltrates	1		.003	1		.004
Diffuse infiltrates	10.8	2.22-52.544		12.285	2.259-66.812	
Assisted microbiological and serological analysis						
Negative	1		.000	1		.000
Positive	5.536	2.512-12.2		6.094	2.539-14.627	

ISD, identical sibling donor; PLT, platelets; FB, flexible bronchoscopy; CT, computed tomography.

serological analyses (62/86, 72%) were associated with a higher likelihood of obtaining a positive FB result than negative results (14/44, 32%; *P* = .000).

3.4 | Clinical results after FB

The overall treatment modification after FB was 61% (n = 79): 73% (n = 66) in the positive FB group and 33% (n = 13) in the negative FB group (*P* = .000). Treatment changes consisted of modifications in the antibiotic (n = 15), antifungal (n = 13), and antiviral (n = 36) medications and the addition of corticosteroids (n = 15). The overall 30-day mortality rate was 32% (n = 42): 34% and 25% in the positive and negative FB groups, respectively (*P* = .334). One patient died of sudden cardiac arrest, while 31 patients died of pneumonia.

4 | DISCUSSION

We firstly used PCR to identify 27 viruses in FB specimens to evaluate pulmonary infiltrates in allo-HSCT patients. In this large single-center study of 130 consecutive patients, the overall diagnostic yield of FB was 58%. FB results prompted a treatment modification in 61% of cases. Eighty-nine percent of positive FB results were infections. A polymicrobial etiology was established in 47% of these cases. Viruses were the most common infectious diagnosis (70%), followed by fungi (48%), bacteria (38%), and *P. jirovecii* (12%).

We attempted to combine the results from BAL, PSB, and TBB in conjunction with PCR to enhance the diagnostic yield of FB compared to other studies, which have reported diagnostic yields of 23%-65%.[4-13] However, the present study did not demonstrate a superior diagnostic yield. Several explanations may account for this outcome. First, the small size of PSB and TBB and the strict definitions for bacterial, viral, and fungal pneumonia could explain why a higher diagnostic yield was not attained in the present study, despite the use of a wide range of molecular diagnostic tests. Second, all patients received empirical antimicrobial therapy prior to FB, which had the potential to decrease the diagnostic yield.

Most of FB results corresponded to infectious microorganisms. Overall, 142 pathogens were considered relevant and were mainly viruses. There were significantly more viruses detected in our study than in previous studies.[7,12,16] Several explanations may account for this outcome. First, bacterial and fungal infections are the primary causes of infection during neutropenic period, while viral infections are more common during the mid- and late-recovery phases after HSCT.[4-6,9,11] Majority of patients in our study were non-neutropenic. Second, compared with previous studies, we used PCR to detect 27 viruses, which greatly improved the diagnosis of viruses and allowed the early administration of appropriate therapies. However, these assays were not available during the previous study periods. The results demonstrate that PCR is a fast and effective diagnostic method for viruses. In our study, CMV was the most common pathogen found, which is in agreement with previous studies,[7,16] followed by RSV and parainfluenza virus.

Aspergillus pneumonia accounted for 62% of all fungal pneumonia and was the most common co-pathogen in the polymicrobial pulmonary infection group (42%), which may correlate with the relatively higher proportion of haplo-HSCT (74%) in our study. In accordance with previous studies, the threshold value for positive BAL GM in our study was 0.5.[6,19] PJP was found in a relatively high percentage (12%). In our study, the diagnosis of PJP was confirmed by PCR in 8/9 (89%) cases, which confirmed that PCR was relatively sensitive for the detection of *P. jirovecii*. In addition to the gold standard method of direct visualization of the pathogen, PCR was employed in the current study for the detection of *P. jirovecii* DNA in BAL and to establish the diagnosis of PJP. Indeed, the direct visualization was positive only in one of nine patients ultimately diagnosed with PJP. The PJP cases with positive PCR and negative smear probably did not represent colonization,

since the characteristic clinical and radiological picture of PJP was observed in high-risk patients. Moreover, a previous study confirmed the PCR assay to be relatively sensitive and highly specific for the detection of *P. jirovecii* (74% and 95%, respectively), with a low false-positive rate and a positive predictive value of 83%.[20]

Bacterial pneumonia was diagnosed in 38% cases. The most striking difference between our study and previous studies on bacterial pneumonia is the significantly high percentage of TB (29%). This result demonstrates that TB remains a significant issue in China, even with the current progress in medical science. Considering the high risk of lung biopsy and the low positive rate of sputum smear, the use of PCR for diagnosing TB in FB specimens is a safe and appropriate method. The 7% incidence of DAH in our observation is consistent with the incidence of 1%-20% reported in the literature.[21,22]

FB results prompted a treatment modification in 61% of cases, which is higher than 29%-51% reported previously.[4-8,10,12,13] The changes most frequently identified were modifications to anti-infection treatments, especially antiviral treatments. The modifications in positive FB groups were significantly higher than negative FB groups, while there was no significant difference in the 30-day mortality rates between two groups. All of these results prove that PCR in FB is a fast and effective diagnostic method for identifying viruses and allows the early administration of appropriate therapies, thereby eliminating the pathogen.

Our study showed that the diagnostic yield of FB for diffuse infiltrates was better than that for focal infiltrates. This is consistent with the findings of Seneviratna et al[10] and Lanino et al.[23] This reason for this result may be that diffuse infiltrates have a greater probability of having an infectious cause and a high pathogen burden than do focal infiltrates. In addition, a notable difference in our study compared with previous reports is that the group with positive results in the assisted microbiological and serological analyses was associated with a higher likelihood of obtaining a positive FB. It may also be explained by a higher probability of having an infectious basis.

The limitations of our study are multifactorial. First, this is a retrospective single-center study. Second, the most important limitation of this study is no consensus regarding the optimal timing for FB in allo-HSCT recipients at our center, which may introduce bias. Third, although we defined treatment modifications as changes introduced within 3 days after FB, we did not have a definitive way to ensure that these changes were all accurate given the retrospective nature of the study. Finally, our study did not represent the incidence of pneumonia following allo-HSCT, because this would require an analysis of all allo-HSCT patients with new pulmonary infiltrates.

In summary, this large single-center study firstly used PCR to identify 27 viruses in FB samples to evaluate pulmonary infiltrates in allo-HSCT population. We confirmed that FB in conjunction with PCR is efficient in the rapid diagnosis and management of pulmonary infiltrates in allo-HSCT patients.

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CONFLICT OF INTEREST

None.

AUTHORS' CONTRIBUTIONS

Xiao-Jun Huang and Lan-Ping Xu: Contributed the study concept and design; Fei-Fei Tang and Xiao-Dong Mo: Performed data analysis; Fei-Fei Tang: Wrote the manuscript; All authors: Participated in providing patients' data.

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