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Fungi identified via next-generation sequencing in bronchoalveolar lavage fluid among patients with COVID-19: a retrospective study

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Abstract

Background The epidemiology of fungi identified via next-generation sequencing in bronchoalveolar lavage fluid among patients with COVID-19 is unknown.

Methods De-identified information, including age, SARS-CoV-2 reads and fungi from bronchoalveolar lavage fluid, were used to analysis.

Results A total of 960 patients with COVID-19 were included. Gender was unknown in 38 patients, and 648 (70.3%) of the rest patients were male. For 876 patients with information on age, their mean ± standard age was 63.4 ± 21.3 years, with the minimum being 0.2 years and the maximum being 101 years. For all the patients, their median [interquartile range] SARS-CoV-2 reads were 26,038 [4421.5, 44,641.5]. The Aspergilli were identified in 159 (16.6%) patients, with *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* in 103 (10.7%), 81 (8.4%) and 17 (1.8%), respectively. The *Mucoraceae* were identified in 14 (1.5%) patients. *Pneumocystis jirovecii* was identified in 65 (6.8%) patients, among whom 12 (18.5%) patients also had Aspergilli. The *Cryptococcaceae* and the *Dematiaceae* were also identified in some patients, including *Cryptococcus* in 11 (1.1%) patients.

Conclusions In bronchoalveolar lavage fluid among patients with COVID-19, the Aspergilli were very commonly identified, as were the *Mucoraceae*, *Pneumocystis jirovecii* and *Cryptococcus* via next-generation sequencing.

Keywords Next-generation sequencing, Bronchoalveolar lavage fluid, COVID-19, Fungal infection, Aspergilli, *Mucoraceae*, *Pneumocystis jirovecii*, *Cryptococcus*

Background

In patients with coronavirus disease 2019 (COVID-19), fungal co-infection is associated with a significant increase in morbidity and mortality, especially in critically ill patients in intensive care units (ICUs) [1]. Fungal co-infection was most commonly identified in respiratory tract and blood. The diagnosis of proven invasive pulmonary aspergillosis (IPA) and mucormycosis requires microbiologic or histopathologic evidence. The former is more commonly used and more feasible than the latter. However, a microbiologic examination is mainly based on culture, which is time-consuming and is of low sensitivity. Another microbiologic method is molecular analysis of DNA sequences of fungi in bronchoalveolar lavage fluid (BALF) [2]. As far as we know, limited information on DNA sequences of fungi in BALF among patients with COVID-19 is available. We aimed to fill this gap by

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conducting a retrospective study on fungi identified by next-generation sequencing (NGS) of pathogens in BALF among patients with COVID-19.

Methods

When caring for intubated patients with COVID-19, if superinfection including fungal infection is suspected and microbiologic culture is not sufficient to explain the condition or not efficient enough, treating physicians sometimes ask for the permission from a patient's next of kin and provide demographic information and a BALF specimen to an NGS company. The patient's next of kin pays for the test. A PDF file of the result is sent both to the patient's next of kin and to treating physicians.

This study was approved and consent was waived by the Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (2023-0157-01). De-identified data comprising of age, gender, date of specimen collection, SARS-CoV-2 reads and fungi from BALF were retrospectively collected from critical care physicians who are working in tertiary hospitals and are acquainted with the authors. Patients with COVID-19 and a result of BALF NGS were included. Except for the first result, patients with more than one BALF NGS result were excluded.

Statistical analysis

Age was presented as mean ± standard deviation, and SARS-CoV-2 reads as median [interquartile range]. Qualitative data were presented as count (percentage). No comparison or regression was conducted.

Results

From December 13th, 2022 to January 5th, 2023, 960 patients with COVID-19 were included, with one record for each patient and they were from 1 autonomous region, 2 centrally-administered municipalities and 18 provinces of China's mainland.

Gender was unknown in 38 patients, and 648 (70.3%) of the rest patients were male. For 876 patients with information on age, their mean ± standard age was 63.4 ± 21.3 years, with the minimum being 0.2 years and the maximum being 101 years (Table 1). Among these patients, 53 (6.1%) patients were below 18 years, and 521 (59.5) patients no less than 65 years, including 195 (22.3%) no less than 80 years.

For all the patients, their median [interquartile range] SARS-CoV-2 reads were 26,038 [4421.5, 44,641.5]. The Aspergilli were identified in 159 (16.6%) patients, with *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* in 103 (10.7%), 81 (8.4%) and 17 (1.8%), respectively (Table 2). In patients with *Aspergillus*, *Aspergillus fumigatus* was identified in 64.8% of patients, and *Aspergillus*

Table 1 Age and gender of 960 patients included

Demographic information	Calculated values
Gender (n = 922)	
Male	648 (70.3%)
Age (n = 876)	
Mean ± standard deviation, years	63.4 ± 21.3
Minimum, years	0.2
Maximum, year	101
Age < 18 years	53 (6.1%)
Age < 6 years	19 (2.2%)
Age < 1	7 (0.8%)
Age ≥ 65	521 (59.5%)
Age ≥ 80	195 (22.3%)

Table 2 Fungi identified by next-generation sequencing in BALF from 960 patients with COVID-19

Family/genus/species of fungi from BALF	Number of patients (%)
Moniliaceae	159 (16.6%)
Aspergillus	159 (16.6%)
Aspergillus fumigatus	103 (10.7%)
Aspergillus flavus	81 (8.4%)
Aspergillus niger	17 (1.8%)
Aspergillus terreus	7 (0.7%)
Aspergillus hominis	2 (0.2%)
Aspergillus tubingenensis	1 (0.1%)
Mucoraceae	14 (1.5%)
Rhizopus	12 (1.3%)
Rhizopus oryzae	7 (0.7%)
Rhizopus microspores	5 (0.5%)
Rhizomucor	3 (0.3%)
Rhizomucor pusillus	3 (0.3%)
Pneumocystidaceae	65 (6.8%)
Pneumocystis	
Pneumocystis jirovecii	65 (6.8%)
Cryptococcaceae	14 (1.5%)
Trichosporon	
Trichosporon asahii	3 (0.3%)
Cryptococcus	
Cryptococcus neoformans	11 (1.1%)
Dematiaceae	1 (0.1%)
Exophiala	
Exophiala dermatitidis	1 (0.1%)
Candida	381 (39.7%)
Candida albicans	303 (31.6%)
Candida tropicalis	58 (6.0%)
Candida krusei	11 (1.2%)
Candida glabrata	86 (9.0%)
Candida parapsilosis	1 (0.1%)

COVID-19 coronavirus disease 2019, *BALF* bronchoalveolar lavage fluid

flavus in 50.9%. Two *Aspergillus* species were identified in 38 (4.0%) patients, and three in 7 (0.7%) patients. The *Mucoraceae* were identified in 14 (1.5%) patients, with *Rhizopus* in 12 (1.3%) and *Rhizomucor* in 3 (0.3%) patients. Two *Mucoraceae* species were identified in 1 patient. Both species of *Aspergillus* and *Mucoraceae* were identified in 11 (1.1%) patients. *Pneumocystis jirovecii* was identified in 65 (6.8%) patients, among whom 12 (18.5%) patients concomitantly had Aspergilli. The *Cryptococcaceae* and the *Dematiaceae* were also identified in some patients, including *Cryptococcus* in 11 (1.1%) patients (Table 2).

Discussion

As far as we know, our study was the first large sample-sized one depicting fungi identified by next-generation sequencing in BALF among patients with COVID-19. Different families of fungi, including *Moniliaceae*, *Mucoraceae*, *Pneumocystidaceae*, and *Cryptococcaceae* were identified.

Most fungi are opportunistic pathogenic microorganisms, but invasive fungal infection (IFI) complicates the clinical course of critically ill patients and is life-threatening [1]. Respiratory viruses, like influenza and SARS-CoV-2, damage the respiratory system directly, facilitating fungal infection in both immunocompromised and non-immunocompromised patients [3]. Furthermore, SARS-CoV-2 causes a decrease of T-cell, especially in critically ill patients [4]. Treating COVID-19 with dexamethasone and IL-6 receptor antagonists is beneficial in some patients, but is detrimental in other patients, leading to superinfections [3].

IFI is time-sensitive and should be treated promptly, but its timely diagnosis is hard to reach. Generally, host factors, clinical factors (especially radiological findings), and microbiological evidence are used to diagnose fungal infection. IPA is associated with impaired immune function due to neutropenia, hematological or oncological malignancy, stem cell or solid organ transplantation, prolonged use of corticosteroids, immunosuppressive or cytotoxic treatment, and inborn or acquired immunodeficiency [1, 3]. Besides these immunosuppressive conditions, mucormycosis is also associated with poorly controlled diabetes mellitus and malnutrition [5]. Due to a high prevalence of SARS-CoV-2 infection, many critically ill patients with COVID-19 lack those host factors. Radiological presentations in patients with COVID-19 are manifold and non-specific, resembling those of IPA and probably overshadowing those of IPA from non-COVID-19 patients with typical host factors. Mucormycosis begins with the inhalation of sporangiospores ubiquitous in the environment. During mechanical ventilation, sporangiospores could go directly into lungs. In spontaneous breathing patients, sporangiospores infect the nasal turbinates, and invade hard palate, paranasal sinuses, orbit, and brain. Besides pulmonary involvement, the *Mucoraceae* causes rhino-orbital and cerebral damages aggressively [6, 7].

The gold standard in the confirmation of invasive pulmonary fungal infection is lung biopsy culture and tissue microscopy [1, 3]. The biopsy of infected lungs is a high-risk procedure, which is not always feasible. For one thing, the clinical course of critically ill patients with COVID-19 is usually long-lasting, refuting repeated biopsies. For another, medical resources are overwhelmed in face of the extremely large patient population. Bronchoscopy and BALF are more obtainable than lung biopsies and are considered as best alternatives. Positive BALF culture and DNA sequences are used to diagnose IFI. PCR testing of BALF is also used in diagnosing IPA, which is considered at least similarly specific to that of galactomannan testing [1, 8]. However, PCR testing of BALF is not considered a diagnostic criterion by AspICU and BM-Asp ICU [9, 10].

Different families of fungi infect lungs of COVID-19 patients. NGS detects multiple fungi at the same time, while confirming SARS-CoV-2 infection. In a study of non-ARDS and non-COVID-19 patients, Zhan et al. found that in 28 BALF NGS *Aspergillus*-positive traditional immunocompromised patients, 23 (82.1%) patients met the diagnosis of IPA using European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [11]. As far as we know, the test performance of BALF NGS in critically ill COVID-19 patients has not been studied. Whether BALF NGS results should be incorporated into the diagnosis of IFI in different patient populations needs further exploration.

Some findings are worth noting. The prevalence of Aspergilli identified in our study was 16.6%, which is similar to proven and probable COVID-19-associated pulmonary aspergillosis from a previous study. During the first wave of SARS-CoV-2 infection, Gangneux et al. found that 76 (15%) patients met the European Confederation of Medical Mycology and International Society for Human and Animal Mycology criteria for proven and probable pulmonary aspergillosis in mechanically ventilated COVID-19 patients [12]. *Aspergillus fumigatus* constitutes 68% of *Aspergillus* species identified in BALF, which is almost identical to 66% reported by Apostolopoulou et al. [13]. However, the proportion of *Aspergillus flavus* is much higher in our study. The prevalence of mucormycosis in critical patients with COVID-19 in ICUs was 0.3%-0.8% [14]. During the second wave of COVID-19 epidemic in India, mucormycosis surged [11].

In accordance with previous studies, genus *Rhizopus* is the most commonly identified in humans [15, 16].

Another interesting finding of our study is that *Pneumocystis jirovecii* was identified in 65 (6.8%) patients. Bretagne et al. found that in 244 patients with COVID-19 from intensive care units, *Pneumocystis jirovecii* was identified in 17 (7.0%) patients using PCR assays, and 10 (58.8%) were immunosuppressive [17]. Alanio et al. found that *Pneumocystis jirovecii* PCR was positive in 10 of 108 (9.3%) critically ill HIV-negative COVID-19 patients, 4 of these 10 patients were given a prophylactic regimen of co-trimoxazole and EORTC/MSG criteria of IPA fulfilled in 5 of these 10 patients [18]. The mortality of *Pneumocystis jirovecii* positive critically ill patients with COVID-19 was about 30% in both studies, which is lower than that of patients with IPA [17, 18].

Cryptococcus was identified in 11 (1.1%) patients in our study. *Cryptococcus* can infect blood, lung and meninges concurrently or after recovery in immunocompromised or immunocompetent patients with COVID-19, and the infection may also disseminate [19]. The clinical and radiological presentations of COVID-19 may also overshadow those of Cryptococcus infection, leading to the under-recognition of *Cryptococcus* pulmonary infection. [20] Although the findings of *Candida* were also presented in Table 2, *Candida* airway colonization is common in critically ill patients with COVID-19, but *Candida* pneumonia is rare [12, 17, 20].

This study has several limitations. First, the clinical course and prognosis were unavailable. Second, past medical history and laboratory test results were unavailable, making it hard to interpret the relationship among fungal findings, SARS-CoV-2 infection and preceding immune conditions. Third, although we believe most if not all of BALF in this study was obtained in mechanically ventilated critically ill patients, we cannot prove it. Fourth, the results from different NGS companies were retrospectively used, and NGS workflows may differ. Fifth, false-positive next-generation sequencing (NGS) results can occur [21]. Contamination occurs as common microbial cultures. Environmental contamination usually comes from the air in the room. Other sources of contamination include the specimen container, the bronchoscope, handling of BALF samples or even the reagents used. Secretions from the upper respiratory tract can transfer colonized microorganisms to the lower respiratory tract, which can then be detected by NGS.

Conclusions

In bronchoalveolar lavage fluid among patients with COVID-19, the Aspergilli were very commonly identified, as were the *Mucoraceae*, *Pneumocystis jirovecii* and *Cryptococcus* via next-generation sequencing.

Abbreviations

BALF	Bronchoalveolar lavage fluid
COVID-19	Coronavirus disease 2019
EORTC/MSG	European Organization for Research and Treatment of Cancer/Mycoses Study Group
ICU	Intensive care unit
IFI	Invasive fungal infection
IPA	Invasive pulmonary aspergillosis
NGS	Next-generation sequencing

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Author contributions

XY, XG, and YS designed the study. XG, JX, and YS contributed to the implementation of the study and data collection. XY, XG, and HZ wrote and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the declaration of Helsinki and national and institutional standards. This study was approved and consent was waived by the Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (2023-0157-01).

Consent for publication

Not applicable.

Competing interests

The authors had an experience collaborating with NGS companies to identify pathogens while treating critically ill patients. All authors declare that they have no other competing interests.

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