

Risk Factors of Fungal Growth in Sputum Culture from Severe and Critical COVID-19 Patients

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ABSTRACT

Patients infected with Coronavirus Disease (COVID)-19, especially those with comorbidities, immunosuppressive treatment, intubated, and on ventilators, are more likely to contract fungal infection. This study aimed to describe the profile of fungal growth in sputum culture from severe and critical COVID-19 patients, and to determine the association between the fungal growth with the patient's outcome and the association between several risk factors with the fungal growth. A retrospective case-control study was carried out at Husada Utama Hospital, Surabaya. This study samples were 119 patients; fungal growth on sputum culture was seen in 64 (53.8%) patients; the three types of fungi with the highest frequency were *Cryptococcus neoformans* found in 23 (19.3%) patients, *Candida tropicalis* found in 11 (9.2%) patients and *Candida dubliniensis* found in 10 (8.4%) patients. There was no significant association between fungal growth and patient mortality with a p-value of 0.940 ($p > 0.05$), also between the patient's history of diabetes mellitus and fungal growth ($p = 0.496$) and between corticosteroid treatment and fungal growth ($p = 0.168$). Still, there was a significant association between the use of ventilators in patients and fungal growth with a p-value of 0.001 ($p < 0.05$). A significant association was also found between IL-6 inhibitor (Tocilizumab) treatment and fungal growth with a p-value of 0.003 ($p < 0.05$). The most common fungi found in this study with the highest frequency was *Cryptococcus neoformans*. There was an association between two risk factors (the use of a ventilator and tocilizumab treatment) and fungal growth.

Keywords: Fungi, COVID-19, sputum culture, ventilator, Tocilizumab

INTRODUCTION

Coronavirus Disease 19 (COVID-19) is an infectious disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus, resulting in mild to severe respiratory illness. Older people and those with underlying diseases like diabetes, cardiovascular disease, or cancer are more likely to develop severe illness. The virus is spreading from the mouth or nose of an infected person as small liquid particles, with sizes ranging from larger respiratory droplets to smaller aerosols.¹

Patients infected with COVID-19 are prone to bacterial and fungal coinfection. Patients with comorbidities, immunosuppressive states, current intubation, and mechanical ventilation are more likely to contract fungal infections.²

Severe and critical COVID-19 treatment, such as mechanical ventilation, corticosteroid, and Tocilizumab treatment, can increase the risk of bacterial and fungal infection. Severe COVID-19 disease is associated with increased proinflammatory cytokines, interleukins (IL-1, IL-6), Tumor Necrosis Factor-alpha (TNF-alpha), decreased number of

Cluster of Differentiation 4 (CD4) and CD8 T cells, which eventually lead to the increased susceptibility to fungal infections.³⁻⁵

This study aimed to describe the profile of fungal growth in sputum culture from severe and critical COVID-19 patients and to determine the association between the fungal growth and the patient's outcome and the association between several risk factors and fungal growth.

METHODS

A retrospective case-control study was carried out at Husada Utama Hospital, a private COVID-19 referral hospital in Surabaya. Data of patient characteristics (gender, age), comorbidities illness (diabetes, and other comorbidities), category of COVID-19 clinical disease, the use of mechanical ventilation, immunosuppressive treatment (corticosteroid), IL-6 inhibitor treatment (Tocilizumab), microbiological data from sputum culture, and the outcomes after hospitalization (mortality) were collected from medical records between January 2020 to December 2021.

All COVID-19 patients who underwent sputum cultures and were categorized as severely and critically ill were included. A Chi-Square test was used to measure the significance association between two categorical data groups at the significance level of 0.05. The odd ratio was also used to determine the probability of fungal growth in several risk factors (diabetes mellitus comorbidity, ventilator use, corticosteroid treatment, and Tocilizumab treatment).

The microbiological samples were sent and cultured in a Granostic Diagnostic Center, a referral laboratory in Surabaya. The sputum samples were collected by endotracheal aspiration in intubated patients and by oropharyngeal aspiration for non-intubated patients. Fungal identification and antifungal sensitivity tests were carried out on the VITEK2 Compact device.

The severity of COVID-19 was categorized based on The Guidelines for the Management of COVID-19 (Pedoman Tatalaksana COVID-19) published by Perhimpunan Dokter Paru Indonesia (PDPI), Perhimpunan Dokter Spesialis Kardiovaskular Indonesia (PERKI), Perhimpunan Dokter Spesialis Penyakit Dalam Indonesia (PAPDI), Perhimpunan Dokter Anestesiologi and Terapi Intensif Indonesia (PERDATIN), and Ikatan Dokter Anak Indonesia (IDAI), first, second and third edition.⁶⁻⁸

Ethical eligibility approval was obtained from the Health Research Ethics Commission, Faculty of Public Health Universitas Airlangga, No. 60/EA/KEPK/2022.

RESULTS AND DISCUSSIONS

This study collected data on 3513 COVID-19 patients from January 2020 to December 2021, consisting of 124 patients who underwent sputum cultures and 119 of the 124 patients classified as severe and critical COVID-19. These research subjects were 119 patients who underwent sputum cultures and were classified as severe and critical COVID-19.

Table 1 shows that from 119 patients who underwent sputum culture, 72 (60.5%) had comorbidities, and 71 (59.7%) weren't diabetics. One hundred two (85.7%) out of 119 patients in this study died, and only 17 (14.3%) patients recovered; 110 (92.4%) patients were on ventilators, and only 9 (7.6%) patients did not use ventilators. Of the patients in this study, 112 patients (94.1%) received corticosteroid treatment, 65 (54.6%) patients received Tocilizumab treatment, and 54 (45.4%) patients didn't receive Tocilizumab.

An overview of sputum culture results from 119 patients with severe and critical COVID-19 are listed in Table 2, showing that most patients had fungal

Table 1. Clinical characteristics of research subjects

Profile	Frequency	(%)
Comorbidities		
No	47	39.5
Yes	72	60.5
Diabetes Mellitus (DM)		
Non-DM	71	59.7
DM	48	40.3
Outcome		
Recovery	17	14.3
Death	102	85.7
The use of the ventilator		
No	9	7.6
Yes	110	92.4
Corticosteroid treatment		
No	7	5.9
Yes	112	94.1
Tocilizumab treatment		
No	54	45.4
Yes	65	54.6

Source: analyzed data (2023)

Table 2. Fungal growth in sputum culture

Sputum Culture Result	Frequency	(%)
Fungal growth		
None	55	46.2
Positive	64	53.8
<i>Candida glabrata</i>	8	6.7
<i>Cryptococcus neoformans</i>	23	19.3
<i>Candida tropicalis</i>	11	9.2
<i>Candida albicans</i>	5	4.2
<i>Candida ciferrii</i>	2	1.7
<i>Candida dubliniensis</i>	10	8.4
<i>Candida guilliermondii</i>	3	2.5
<i>Candida parapsilosis</i>	2	1.7

Source: analyzed data (2023)

growth. Fungal growth on sputum culture was observed in 64 (53.8%) patients; the most common three types of fungi with the highest frequency were *Cryptococcus neoformans* found in 23 (19.3%) patients, *Candida tropicalis* found in 11 (9.2%) patients and *Candida dubliniensis* found in 10 (8.4%) patients. The finding of the high frequency of *Cryptococcus neoformans* growth in the sputum culture of COVID-19 patients is fascinating; whether this *Cryptococcus neoformans* has caused pulmonary cryptococcosis, which can cause respiratory failure, as reported by Sharma *et al.* about a rare case of COVID-19-associated pulmonary cryptococcosis in January 2022.⁹ About 50% of cases are asymptomatic in immunocompetent patients, but rarely asymptomatic in immunocompromised patients.

Even 33% of them experienced acute respiratory failure.¹⁰ Another data from hospital antifungal antibiogram 2020-2021 showed a very low (29.17%) sensitivity of *Cryptococcus neoformans* to Amphotericin B in the hospital in which the data in this study were collected.

Candida is an opportunistic pathogen that can cause superficial infections, (which include cutaneous and mucosal candidiasis) and invasive systemic infections. Although the definitive test of *Candida pneumonia* is lung biopsy with a very low incidence rate, *Candida* in respiratory specimens is usually regarded as colonization.¹¹⁻¹³ Hughes *et al.* mentioned that *Candida spp.* was found in 21.4% of respiratory sample cultures in the UK and suggested its possibility to represent oropharyngeal thrush or normal flora rather than pulmonary candidiasis, while other publications excluded the growth of *Candida spp.* in respiratory cultures as an indicator the presence of pneumonia.^{14,15} Pendleton *et al.* mentioned publications about the synergistic effect between *Candida* species and bacterial pathogens, although the mechanism of how *Candida* can potentiate bacterial pneumonia remains incompletely understood. It was hypothesized that the presence of *C.albicans* in the airways can induce an immune response that inhibits the regular antibacterial activity of host immune cells, allowing bacterial pathogens to evade clearance and initiate infection.¹⁶ Additional data in this study showed that 53 (47.9%) from 64 samples with fungal growth also had bacterial growth, and 36 (31.1%) from 55 samples with no fungal growth had bacterial growth, which was predominated by *Acinetobacter baumannii* (16 out of 36 samples).

Table 3 analysis shows no significant association between fungal growth in severe and critical

COVID-19 patients undergoing sputum culture and patient mortality (p-value of 0.940 > 0.05). Although many publications mentioned that fungal infections increase mortality and can potentiate bacterial pathogens, this study found no significant association between fungal growth and patient mortality.¹⁶

Several risk factors associated with the presence of fungal growth were analyzed in this study. It was later found out that a decreased immune response might reduce the patient's immune system and lead to a greater risk of fungal growth. Although risk factors such as DM, ventilator use, corticosteroid, and Tocilizumab treatment were mentioned in this study, there was no significant association between DM and corticosteroid treatment with fungal growth. However, there was a significant association between ventilator use and Tocilizumab treatment, as seen in Tables 4, 5, 6, and 7.

Table 4 also shows no significant association between the patient's history of DM in severe and critical COVID-19 patients undergoing sputum culture and fungal growth (p-value of 0.496 > 0.05). Negm *et al.*'s study also showed that DM was not associated with fungal coinfection but rather with poor diabetic control.¹⁷ Contrastingly, this study did not differentiate between uncontrolled and controlled DM.

Table 5 analysis shows a significant association between the use of ventilators in patients with severe and critical COVID-19 patients and fungal growth (p-value of 0.0007628 <0.05). Unfortunately, a cohort study was not performed to identify Ventilator Associated Pneumonia (VAP) since it was reported in a previous publication by Kubin *et al.* that Hospital Associated Infection (HAIs) occurred in 12% of COVID-19 cases and fungi caused 19% of them.¹⁵ Rouyer *et al.* also mentioned that VAP occurred in

Table 3. Fungal growth and patient's outcome

Fungal growth	Outcome		Total n (%)
	Recovery n (%)	Death n (%)	
No fungal growth	8 (14.5%)	47 (85.5%)	55 (100%)
Fungal growth	9 (14.1%)	55 (85.9%)	64 (100%)
Total	17 (14.3%)	102 (85.7%)	119 (100%)

Note: Chi-Square = 0.006; p-value = 0.940; OR (95% CI) = 1.040 (0.372 – 2.910) Source: analyzed data (2023)

Table 4. Diabetes mellitus and fungal growth

Diabetes Mellitus	Fungal Growth		Total n (%)
	Yes n (%)	No n (%)	
With DM	24 (50%)	24 (50%)	48 (100%)
No DM	40 (56.3%)	31 (43.7%)	71 (100%)
Total	64 (53.8%)	55 (46.2%)	119 (100%)

Note: Chi-Square = 0.463; p-value = 0.496; OR (95%CI) = 0.775 (0.372 – 1.616) Source: analyzed data (2023)

Table 5. The use of a ventilator and fungal growth

The Use of Ventilator	Fungal Growth		Total n (%)
	Yes n (%)	No n (%)	
With ventilator	64 (58.2%)	46 (41.8%)	110 (100%)
Without ventilator	0 (0.0%)	9 (100%)	9 (100%)
Total	64 (53.8%)	55 (46.2%)	119 (100%)

Note: Chi-Square = 11.33; p-value = 0.0007628; OR (95%CI) = can't be analyzed Source: analyzed data (2023)

Table 6. Corticosteroid treatment and fungal growth

Corticosteroid Treatment	Fungal Growth		Total n (%)
	Yes n (%)	No n (%)	
Yes	62 (55.4%)	50 (44.6%)	112 (100%)
No	2 (28.6%)	5 (71.4%)	7 (100%)
Total	64 (53.8%)	55 (46.2%)	119 (100%)

Note: Chi-Square =1.9016; p-value = 0.167896; OR (95%CI) =3.1 (0.5769-16.66) Source: analyzed data (2023)

Table 7. IL-6 inhibitor treatment and fungal growth

IL-6 inhibitor (Tocilizumab) Treatment	Fungal Growth		Total n (%)
	Yes n (%)	No n (%)	
Yes	43 (66.2%)	22 (33.8%)	65 (100%)
No	21 (38.9%)	33 (61.1%)	54 (100%)
Total	64 (53.8%)	55 (46.2%)	119 (100%)

Note: Chi-Square = 8.8211; p-value = 0.002978 OR (95%CI) = 3.071 (1.45– 6.505) Source: analyzed data (2023)

more than 50% of COVID-19 patients using mechanical ventilators.¹⁸

Table 6 shows no significant association between corticosteroid treatment and fungal growth in severe and critical COVID-19 patients undergoing sputum culture (p-value of 0.167896 > 0.05).

A study by Li and Denning suggests that corticosteroid therapy is a risk factor for fungal infections in COVID-19 patients and can worsen outcomes, in contrast to this study.¹⁹

Table 7 analysis shows a significant association between IL-6 inhibitor (Tocilizumab) treatment and fungal growth in severe and critical COVID-19 patients undergoing sputum culture (p-value of 0.002978 < 0.05). The OR value was 3.071, which indicates that patients receiving IL-6 inhibitor (Tocilizumab) treatment are likely to have fungal growth in their sputum culture. This finding was by the previous publications, which mentioned that Tocilizumab therapy showed a significantly increased risk of fungal coinfections in COVID-19 patients.²⁰⁻²²

Tocilizumab is a recombinant humanized anti-IL-6 receptor monoclonal antibody approved by the Food and Drug Administration (FDA) for use in hospitalized adults with COVID-19 who require conventional oxygen, High-Flow Nasal Cannula (HFNC) oxygen, Non-Invasive Ventilation (NIV), or mechanical ventilation. Tocilizumab should be administered intravenously for the treatment of COVID-19. It belongs to the class of IL inhibitors and is used as immunosuppressants.^{23,24}

CONCLUSIONS AND SUGGESTIONS

The highest frequency of fungal growth in sputum culture in severe and critical COVID-19 patients was *Cryptococcus neoformans*, an environmental fungus and opportunistic pathogen in immunocompromised patients. A total of 4 risk factors (DM, the use of ventilator, corticosteroid, and Tocilizumab treatment) were analyzed in this study, and a significant association was found between the use of ventilator and Tocilizumab treatment and fungal growth in severe and critical COVID-19 patients undergoing sputum culture.

Because some publications mentioned that candidiasis can potentiate bacterial pathogens, research was recommended to analyze the association between candidiasis and the presence of bacterial pathogens in respiratory sample cultures and the association between antifungal treatment and decreased susceptibility to bacterial pneumonia.

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