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Research Article

Seroprevalence of Galactomannan *Aspergillus* in COVID-19 patients in Jakarta: A challenge in the pandemic commotion

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ABSTRACT

COVID-19-associated pulmonary aspergillosis (CAPA) is a severe complication of COVID-19 due to *Aspergillus* infection. However, the clinical manifestation and radiology findings are not typical and require measurement of *Aspergillus* galactomannan (GM) level to assist in the diagnosis of CAPA. We evaluated the positivity rate of *Aspergillus* GM level among COVID-19 patients and investigated the correlation between the GM level and the age of patients. This retrospective observational study compiled the GM results from COVID-19 patients' sera during the early period of the COVID-19 pandemic from March 2020 to December 2021. All samples underwent an ELISA test to measure *Aspergillus* GM level. There were 96 patients enrolled in this study. The positivity rate was 33%, with the highest (56%) percentage of positivity observed in the age group 40-59 in the positive *Aspergillus* GM group. The measurement of *Aspergillus* GM level among COVID-19 patients with risk factors of CAPA is critical to establish CAPA diagnosis. The availability of *Aspergillus* GM test in Indonesia is still limited and this issue needs to be addressed by all healthcare experts and stakeholders.

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INTRODUCTION

Aspergillus spp. are ubiquitous fungi that can be found everywhere in the environment. In healthy individuals, inhaled *Aspergillus* conidia will completely be eliminated from the respiratory system. Meanwhile, in individuals with impaired immune systems or certain underlying diseases, *Aspergillus* conidia can colonize and even invade the human respiratory tract, causing invasive pulmonary aspergillosis (IPA). Traditional risk factors of IPA include prolonged neutropenia in hemato-oncology patients, organ transplant, or immunosuppressive agent usage. However, non-traditional risk factors could be found in ICU patients with critical illness, prolonged usage of antibiotics or systemic corticosteroids, chronic obstructive pulmonary disease (COPD), severe influenza, etc (Apostolopoulou et al., 2020; Lai & Yu, 2021).

Impaired mucociliary clearance, local immune paralysis, and respiratory epithelial damage may facilitate *Aspergillus* invasion which became key pathophysiological factors for IPA (Latgé & Chamilos, 2020). Severe COVID-19 is an important risk factor for increasing the frequency of IPA during the pandemic.(Lamoth et al., 2020) The mortality rate of COVID-19 was high in Jakarta during the peak pandemic period *(*Rozaliyani et al., 2020). COVID-19-associated pulmonary aspergillosis (CAPA) became a new challenge that complicates patient management. (Machado et al., 2021). The incidence of CAPA is reported to occur in 3.3% - 33.3% of ICU patients. The failure to timely identify and manage the infection will lead to a 28.5% - 100% mortality rate. (Machado et al., 2021)

A high index of suspicion is required to diagnose CAPA since *Aspergillus* superinfection is difficult to differentiate from severe COVID-19 based on clinical or imaging findings. It is reported as an important complication of COVID-19 in critically ill hospitalized patients. Failure to diagnose CAPA most likely resulted in excessive mortality, as therapy was often inadequate. Certain strategies are necessary to determine the incidence, clinical characteristics, and outcomes of CAPA during critical times (Thompson et al., 2020).

Galactomannan (GM) is a polysaccharide component of *Aspergillus* cell wall that is released into body fluids in the early stages of fungal invasion. Detection of GM *Aspergillus* is widely used as a diagnostic tool in invasive aspergillosis (Lamoth, 2016). The GM detection from bronchoalveolar lavage (BAL) fluid is the most sensitive test for invasive aspergillosis in ICU patients. (Donnelly et al., 2020; Ullmann *et al*., 2018) However, bronchoscopy procedures are not routinely performed in patients with COVID-19 due to the risk of disease transmission. In certain situations, GM serum detection combined with other tests becomes more feasible for CAPA diagnosis. (Koehler et al., 2020)

The difficulty of CAPA diagnosis is a tremendous challenge in resource-limited settings. The availability of GM *Aspergillus* detection and mycology facilities is very limited. It causes the diagnostic criteria for CAPA is still a drawback to fulfill. We aimed to evaluate the results of GM detection in severe COVID-19 patients as an initial study to consider its role in diagnosing CAPA in Indonesia.

METHODS

This retrospective observational study compiles the GM results from COVID-19 patients' sera during the early period of the COVID-19 pandemic from March 2020 to December 2021. The GM detection was conducted at the Parasitology Laboratory, Faculty of Medicine

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Universitas Indonesia (FMUI). Almost all patients had a severe COVID-19 pneumonia history and were treated in several hospitals in Jakarta and surrounding cities. The patients' sera were taken for GM detection between the time range 0-48 hours, meanwhile, the sera were stored in a -20 freezer. The inclusion criteria were the sera that came from patients with COVID-19 to the Parasitology Laboratory FMUI within the study period. The exclusion criteria were samples with incomplete demographic information such as age and gender. The GM detection was carried out using a double-sandwich ELISA by Platelia *Aspergillus* antigen assay following the manufacturer's instructions (Bio-Rad Laboratories, USA). The positivity threshold was GM optical density index (ODI) > 1.0 according to the standard protocol. This study was part of a previous study on COVID-19 patients in Jakarta and the related co-infections. It was approved by the Health Research Ethics Committee of the FMUI through the certificate of passing ethics review No. 042/UN2.F1/ETIK/PPM.00.02/2021.

The SPSS version 20.0 software (IBM, Armonk, NY, USA) was used to perform the statistical analyses. Categorical data were presented as numbers of cases and percentages. Fisher's exact tests or X^2 tests were used for categorical variables for positive and negative *Aspergillus* galactomannan tests. A p-value of $p<0.05$ was considered statistically significant.

RESULTS

A total of 96 patients with COVID-19 and suspected of having IPA who were examined during the study period were included in this study. There were 68 (71%) males and 28 (29%) females. Most (46%) of patients were in the age group range 60–79 years old. The *Aspergillus* GM level was positive in thirty-two (33%) of 96 patients (table 1).

Of the 32 patients with positive GM results, the proportion based on the age group revealed that 18 (56%) patients were in the age group of 40–59 years, 12 (38%) patients were in the age group of 60–79 years, and another two (6%) patients were >80 years old. Of the 64 patients who had negative GM results, 32 (50%) patients were in the age group of 60–79 years, and 24 (38%) patients were in the age group of 40–59 years. All 4 patients in the age group of 20–40 years showed negative GM results.

The mean level of the *Aspergillus* GM test varied across the age groups. The lowest mean level was observed in the age group 60-79 with a level range of 0.54-0.94. Among the negative GM groups, the mean level ranges from 0.54-0.71. Furthermore, the mean level ranges of the positive GM groups were 1.12-1.78, with the highest mean level (1.78) in the age group of 60-79 years and 40-59 years (Figure 1). However, three patients had a GM index >3.0, comprised of two patients in the age group of 40-59 years, and one patient aged 77 years.

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Table 1. Demographic characteristics of patients

	Total (n=96)	Positive GM (n=32)	Negative GM (n=64)	p-value
Gender				
Female	28 (29%)	11 (34%)	17 (27%)	
Male	68 (71%)	21 (66%)	47 (73%)	0.427
Age Groups				
20-39	4 (4%)	0 (0%)	4 (6%)	0.298
40-59	42 (44%)	18 (56%)	24 (38%)	0.081
60-79	44 (46%)	12 (38%)	32 (50%)	0.247
>80	6 (6%)	2 (6%)	4 (6%)	1.000

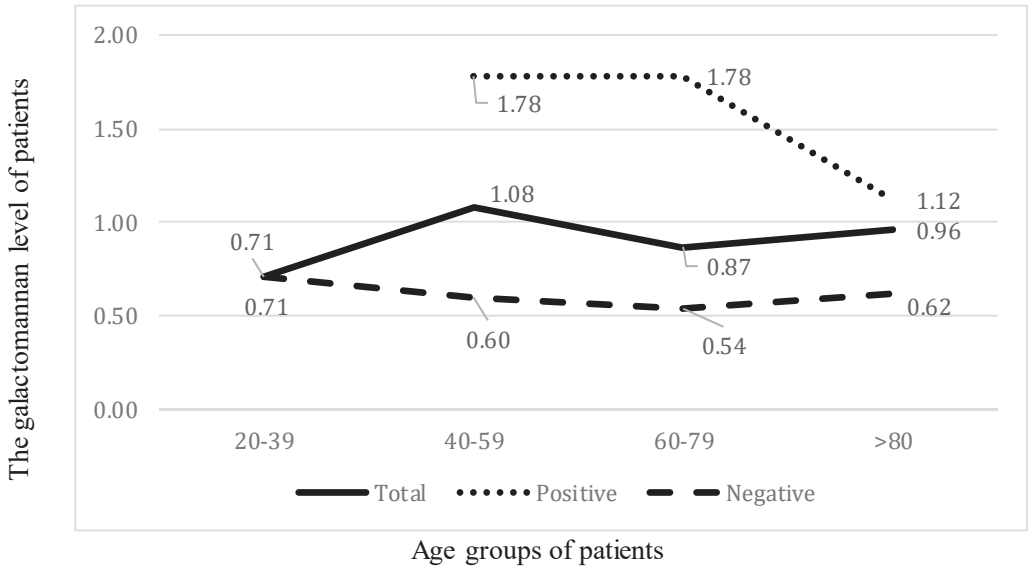

Figure 1. The variety of *Aspergillus* galactomannan means level of COVID-19 patients with suspected invasive pulmonary aspergillosis.

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DISCUSSION

The classic form of IPA is different from invasive aspergillosis which complicates severe COVID-19 known as CAPA. The respiratory epithelium damage will permit *Aspergillus* invasion. Furthermore, lymphopenia with altered activity of macrophages and natural killer cells with associated cytokine imbalances composes an immunosuppressive environment for secondary infections to evolve. The superinfection of aspergillosis and severe COVID-19 comprises a constellation of invasive airway disease and angio-invasive diseases. The condition poses risks associated with poor clearance and killing of *Aspergillus* from the airway. These circumstances will result in virus-associated epithelial damage or inflammation, systemic immunosuppression, and underlying pulmonary disease (Marr et al., 2021; Rutsaert et al., 2020).

The positivity rate of GM in CAPA varied across patient's population. Around one-third of COVID patients showed positive *Aspergillus* GM test in a previous study (Das et al., 2022; Pintado et al., 2021). The rate is similar to our finding, with the positivity rate of *Aspergillus* GM at 33%. The prior study proved that older patients had an increased risk of CAPA (Prattes et al., 2022). This might explain the absence of positive *Aspergillus* GM in the younger age group (20-39). A prior study revealed the median level of *Aspergillus* GM in CAPA patients was 3.5 from BAL (Bartoletti et al., 2020). The highest GM levels in this study were documented in three patients, specifically a GM index of 3.4 in a 49-year-old patient, an index of 3.3 in a 77-year-old patient, and an index of 3.1 in a 51-year-old patient.

The diagnosis of CAPA requires a robust correlation among clinical, radiological, and laboratory data (Koehler et al., 2020). Unfortunately, sufficient clinical and radiological data were not yet available in our laboratory. The integrated data system was not yet available among health services. Whereas adequate data on risk factors, comorbidities, and underlying diseases is pivotal in the diagnosis of CAPA.

The routine use of systemic corticosteroids in severe COVID-19 patients was associated with increasing risk of superinfections and other complications. The common use of corticosteroids in patients with extensive lung damage will be a major risk factor for the development of various infections, including CAPA (Kim et al., 2022). The use of broad-spectrum antibiotics might disrupt the balance of the normal flora from the respiratory epithelium and will also contribute to CAPA development. The aggressive immunomodulatory treatment such as the IL-6 antagonist Tocilizumab in severe COVID-19 patients may also contribute to increased risks of CAPA (Prattes et al., 2022). Diabetes mellitus, obesity, heart disease, hypertension, chronic obstructive pulmonary disease (COPD), and asthma have been reported to correlate with the increased risk of CAPA as well (Calderón-Parra et al., 2022).

Clinical and radiological symptoms are difficult to distinguish from severe COVID-19 or its complications. The growth of *Aspergillus* within clinical material or a positive test for fungal biomarkers does not certainly indicate invasive disease. Radiological abnormalities of CAPA vary and reflect different pathologies. The radiological features consist of well-defined lesions with or without a halo sign, air crescents sign, cavity and wedge, or segmental lobar consolidation. These are classically represented in neutropenic patients. Meanwhile, non-neutropenic patients showed a hint of consolidation, pleural effusion, ground glassing, and tree-in-bud lesions which complicate the diagnosis of CAPA (Donnelly et al., 2020; Koehler et al., 2020; Li & Xia, 2020; Ullmann et al., 2018).

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Sufficient clinical awareness is mandatory for a better diagnosis of CAPA. Any case of severe COVID-19 admitted to ICU, who has a fever with new infiltrates on chest X-ray and not responding to antibiotics or standard treatment, should be considered for CAPA. Further investigations include a combination of GM assay in serum and BALF, and fungal cultures of the lower respiratory samples are highly recommended. However, false negative GM might exist because of the use of the antifungal treatment. It is important to make sure the sample was taken before starting antifungal treatment in patients suspected of CAPA.

Fungal biomarkers play an important role in the diagnostic algorithm of IPA and CAPA. Conventional tests used are GM assay in bronchoalveolar lavage fluid (BALF) or serum. Diagnosis of CAPA still becomes an obstacle due to the risk of aerosol transmission of COVID-19 during a bronchoscopy procedure. Another challenge is the lower sensitivity of serum GM in the non-neutropenic population. The variation in GM performance depends on the use of different cut-off values, the population tested, and the case definition used in the studies. GM detection in bronchoalveolar lavage fluid (BALF) was reported as more sensitive than direct microscopy, fungal culture, or serum GM detection. The concentration of GM in BALF is usually higher than serum GM, being useful for the early diagnosis of CAPA. However, the best cut-off value for the GM index is still being debated. The cut-offs most frequently used were ≥0.5 and ≥1, although results varied widely across studies (Lass-Flörl, Samardzic, & Knoll, 2021).

The procedure for collecting BALF specimens in COVID-19 patients is hazardous, so the use of GM serum is the most feasible, although not ideal. GM detection cannot be used solely for the diagnosis of CAPA but must be combined with clinical and radiological data. The patient's clinical condition, including the COVID severity, underlying diseases, and co-morbidities should also become a particular concern. Moreover, serial GM detection should also be conducted to confirm the diagnosis, assess the disease progression, and evaluation of therapy. This necessity is still a noteworthy obstacle in resource-constrained settings.

The pandemic commotion shocked everyone and led to a lack of preparations. The health facilities were initially focused on the diagnosis and treatment of COVID-19 itself. The efforts to deal with complications were carried out gradually. Insufficient infrastructure and ICU protocols might restrict the diagnostic capabilities when CAPA is concerned. The critically ill nature of the ICU patients and the risk of aerosol generation during the broncho-invasive procedures are other compelling reasons. The treatment should be initiated according to the guidelines when the diagnosis has been confirmed or based on clinical suspicion. The occurrence of CAPA in COVID-19 patients raises new awareness of invasive fungal infection as a complication of COVID-19, particularly in patients with severe or critical illness.

This study has several limitations. First, serum GM detection was only available on one occurrence, whereas serial GM detection was not yet possible due to financial constraints. Second, complete clinical and radiological data were rarely available in our laboratory. Third, GM detection was only available in certain reference laboratories, so the coverage area was also limited. Future study is urgently required to provide a better depiction of CAPA in Indonesia.

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CONCLUSION

Comprehensive data is an important key factor in the CAPA diagnosis. For this reason, a wider collaboration among physicians is mandatory, as well as the ability of an integrated data system. The limited availability of diagnostic tools such as the *Aspergillus* GM test is commonly found in resource-constrained settings such as Indonesia. This may lead to an incapability to show the actual burden of CAPA. The situation is such an iceberg phenomenon that must be solved by all stakeholders, particularly the health authorities, for the better management of CAPA in the future.

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