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COVID-19 associated pulmonary aspergillosis (CAPA) in patients admitted with severe COVID-19 pneumonia: An observational study from Pakistan

Article Summary Line: COVID-19 associated aspergillosis has been reported as a complication of severe COVID-19 pneumonia admitted to ICU in Pakistan.

Running Title: COVID-19 associated aspergillosis (CAPA) from Pakistan

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Abstract:

Background

Invasive aspergillosis is a well-known complication of severe influenza pneumonia with acute respiratory distress syndrome (ARDS). However, recent studies are reporting emergence of aspergillosis in severe COVID-19 pneumonia, named as COVID-19 associated aspergillosis (CAPA).

Methods

A retrospective observational study was conducted in patients with severe COVID-19 pneumonia from February 2020- April 2020. Patients' ≥ 18 years of age with clinical features and abnormal chest imaging with confirmed COVID-19 by RT-PCR for SARS-Cov-2 were included. CAPA was diagnosed based on clinical parameters, radiological findings and mycological data. Data were recorded on a structured proforma and descriptive analysis was performed using Stata ver 12.1.

Results

A total of 147 patients with confirmed COVID-19 and 23 (15.6%) patients requiring ICU admission were identified. *Aspergillus* species were isolated from tracheal aspirates of nine (39.1%) patients and of these five patients (21.7%) were diagnosed with CAPA and four (17.4%) had *Aspergillus* colonization. The mean age of patients with CAPA was 69 years (Median age: 71,

IQR: 24, Range: 51 – 85) and 3/5 patients were male. The most frequent co-morbid was diabetes mellitus (4/5). The overall fatality rate of COVID-19 patients with aspergillosis was 44% (4/9). The cause of death was ARDS in all 3 patients with CAPA and the median length of stay was 16 days (IQR: 10; Range 6-35 days).

Conclusion

This study highlights the need for comparative studies to establish whether there is an association of aspergillosis and COVID-19 and the need for screening for fungal infections in severe COVID-19 patients with certain risk factors.

Keywords: COVID-19 associated aspergillosis, severe COVID-19, Pakistan, invasive aspergillosis, ICU admission

Introduction:

COVID-19 was declared a pandemic by the World Health Organization (WHO) in March 2020¹. Patients with severe COVID-19 pneumonia and acute respiratory distress syndrome (ARDS) may become prone to invasive aspergillosis due to prolonged ICU admission, use of steroids and immunomodulators. Recent reports from Netherlands, Germany and France report invasive aspergillosis in 19%, 26% and 33% patients with severe COVID-19 pneumonia respectively. These studies recommend screening for invasive aspergillosis in severe COVID-19 patients²⁻⁴. An expert panel recently recommends that patients with evidence of *Aspergillus* in serum or bronchoalveolar lavage should be treated with antifungals. The group also recommended need for

more epidemiological data for the new entity COVID-19 associated pulmonary aspergillosis (CAPA)⁵.

Therefore, in this study, we describe the clinical manifestations and outcomes of COVID-19 patients with pulmonary aspergillosis from a tertiary care hospital in Karachi, Pakistan.

Methods:

We conducted a retrospective observational study on PCR positive COVID-19 patients admitted from March 2020 –April 2020 to the Aga Khan University hospital (AKUH), a 700-bedded tertiary care hospital in Karachi, Pakistan. Patients' ≥18 year of both sexes, bilateral pulmonary infiltrates, and confirmed COVID-19 infection based on a positive SARS-CoV-2 RT-PCR were included. COVID-19 associated pulmonary aspergillosis (CAPA) was diagnosed if there were clinical signs and symptoms, an abnormal lung imaging, respiratory specimen culture (bronchoalveolar lavage (BAL), tracheal aspirate or sputum) positive for *Aspergillus* spp. or a positive serum or lower respiratory samples galactomannan index of more than 0.5 and 1.0 respectively in patients who were either not improving from COVID-19 or who worsened after transient improvement of symptoms from COVID-19.

Putative invasive pulmonary aspergillosis (PIPA) was defined as the isolation of *Aspergillus* spp. from any lower respiratory tract specimen, together with meeting three criteria: (1) signs and symptoms (e.g. worsening respiratory failure despite adequate treatment or after transient improvement of symptoms from COVID-19), (2) abnormal imaging and (3) either a) immunocompromised host, e.g. with underlying malignancy or receiving steroids or other immunomodulatory agents, or b) *Aspergillus*-positive smear and culture of tracheal aspirate without any bacteria^{6,7}. Patients were considered colonized if clinical or imaging findings were not suggestive of infection despite positive cultures for *Aspergillus* spp. from a lower respiratory tract specimen.

Laboratory methods:

Nasopharyngeal swabs were processed for detection of SARS-CoV-2 virus by real-time reverse transcriptase polymerase chain reaction (RT-PCR) Cobas® SARS-CoV-2 Qualitative assay for use on the Cobas® 6800/8800 Systems (Roche Molecular Systems, New Jersey, USA). *Aspergillus* species was isolated from the clinical specimen using standard culture methodology. Briefly, a loopful of purulent part of sputa or tracheal aspirate was inoculated on Chocolate agar,

Sheep Blood Agar with colistin, nalidixic acid and MacConkey agar each. An additional 0.5 to 1 ml of the sample was also inoculated on Sabouraud's dextrose agar with chloramphenicol for improved yield of fungal isolates. Gram stain is also routinely performed for all lower respiratory samples to assess their quality and presence of organisms. Isolates were identified based on colony and microscopic morphology. Galactomannan index (GMI) was determined using Platelia Aspergillus®, Bio-Rad Laboratories, Marnes La Coquette, France). Beta-D-Glucan (BG) was performed using (Fungitell®, Associates of Cape Cod, Falmouth, Massachusetts, USA). The serum and respiratory specimen testing was done within a median of 2 days (IQR: 1; Range -2 to 5 days).

Ethical Consideration:

The study was given exemption for ethical approval by the Ethics Review Committee of the Aga Khan University (ERC ref #: 2020-4821-10334).

Data entry and analysis:

Data was collected on a structured proforma on the clinical characteristics, radiological findings, laboratory data and outcomes of COVID-19 patients. Descriptive analysis was performed for demographic features with median and interquartile range (IQR) reported for quantitative variables such as age and length of hospital stay (LOS) and frequencies (percentage) for qualitative variables such as gender, co-morbid conditions, mortality and complications etc. STATA ver 12.1 was used for data analysis.

Results:

A total of 147 patients with COVID-19 were admitted, of these 23 had ARDS⁸ requiring ICU admission. *Aspergillus* species was isolated from nine patients and of these five patients had CAPA (Table 1). None of the patients had a positive GMI and beta-D-glucan was positive in only one patient. Four patients were presumed to have *Aspergillus* colonization; two patients were in recovery phase and in two patients clinical deterioration was attributed to a concomitant bacterial infection.

The median age of COVID-19 patients with CAPA was 71 years (IQR:24; Range 51-85 years). Three out of five patients were male. The most frequent co-morbid was diabetes mellitus (88%) and the most common chest radiograph finding was bilateral interstitial infiltrates in all patients. The time duration between admission and positive culture specimen sent for patients with CAPA was median of 8 days (IQR: 9). Out of nine in whom *Aspergillus* species were isolated from a clinical specimen, seven patients received systemic steroids and five patients received IL-6 antagonist Tocilizumab. Patients were treated for initial 5 to 10 days with hydroxychloroquine and azithromycin during the course of illness. Among those with CAPA, four out of five patients had received systemic steroids and three had received tocilizumab. Four out of five patients had COVID-19 ARDS out of which two required invasive positive pressure ventilation (IPPV) and two patients were managed on noninvasive ventilation (NIV). All 5 patients with CAPA received antifungals. The overall fatality rate of COVID-19 patients with aspergillosis was 44% (n=4) while four patients were discharged, and one is currently under treatment. The cause of death was ARDS in all 3 patients with CAPA and none of the deaths were directly attributable to CAPA. The median length of stay was 16 days (IQR: 10; Range 6-35 days). (Figure 1).

Discussion:

This study demonstrates that invasive aspergillosis is a complication in moderate to severe COVID-19 patients. The risk factors for severe disease in COVID-19 are also the risk factors for aspergillosis including underlying co-morbid like diabetes, prolonged duration of hospitalization and use of immunosuppressive ⁹. ICU aspergillosis is another entity associated with very poor outcomes unless diagnosed and treated early ¹⁰. Three out of five patients with CAPA died in our cohort. This is comparable to the case series from Germany ². Although influenza associated aspergillosis (IAA) is now a well-recognized disease entity, reports of CAPA have just begun to emerge from different parts of the world ^{2,11}. COVID -19 is similar to Influenza with regard to incidence of ARDS but unique with regard to cytokine storm which is being treated with immunomodulators such as IL-6 antagonist Tocilizumab. Tocilizumab has been associated with risk of severe infections particularly tuberculosis¹² and recurrent allergic bronchopulmonary aspergillosis¹³. Although clinically significant disease requiring treatment was present in five out of 9 cases, the Beta-D glucan was elevated in only one case and serum galactomannan was not

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elevated in any of the cases². One hypothesis is that the *Aspergillus* spp may not be invading into the blood vessels to cause release of galactomannan⁵. The finding correlates with the limited literature available on COVID-19 associated aspergillosis suggesting that serum galactomannan may not be the best marker to differentiate between invasive disease and colonization and perhaps BAL galactomannan should be considered wherever possible. However, BAL is usually avoided in view of aerosolization of the virus and high risk of transmissibility to healthcare providers during procedure. Since the radiological findings can overlap between COVID-19 ARDS and invasive pulmonary aspergillosis, seeking microbiological diagnosis is of utmost importance in order to minimize mortality and morbidity due to co-infection. Our study is limited due to small sample size and data from a single center. However, it is imperative to report COVID-19 associated aspergillosis as the case fatality rates have remained higher than that due to Influenza and may be due to co-infections particularly in resource limited settings.

Conclusion

This study highlights the need for comparative studies to establish whether there really is an association of CAPA and COVID-19 and whether COVID-19 patients should undergo screening for fungal infections if they have certain risk factors.

Transparency declaration

Conflict of interest disclosure: All the authors do not have conflict of interests to declare.

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Access to data: Not applicable

Contribution:

N.N designed and performed the research, helped write the manuscript, had full access to all the data. J.F. designed and performed the research and helped write the manuscript. F.M. designed and performed the research and helped write the manuscript. K.J. designed and performed the research, helped write the manuscript, had full access to all the data and had final responsibility for the decision to submit for publication.

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Table 1: Patients with severe COVID-19 with Aspergillosis

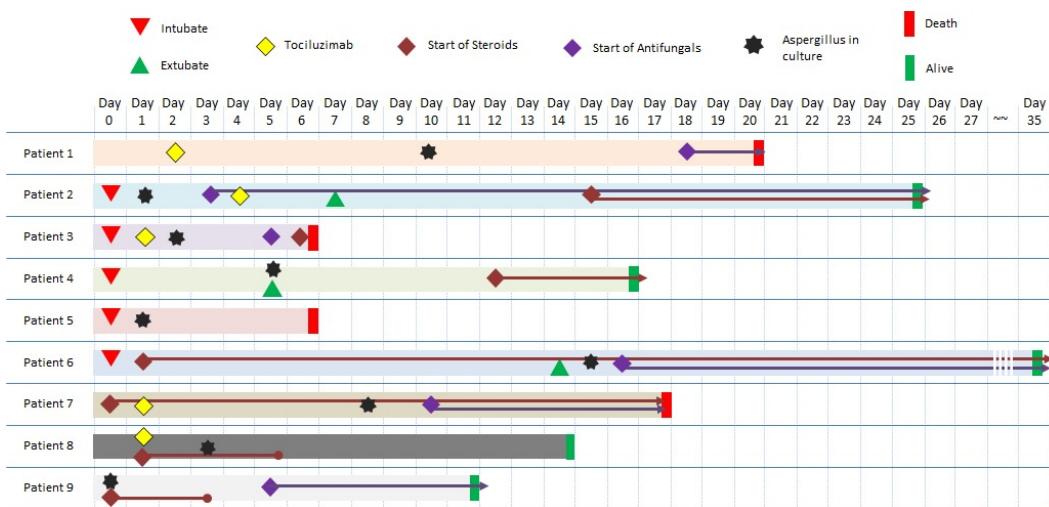
No	Age /Sex	Associated diseases	<i>A.</i> species (number of samples culture positive)	Chest radiograph finding	GMI /BDG pg/ml	Other organisms	Complication	Treatment	Length of stay in days	Outcome	CAPA	Time from COVID-19 to CAPA/colonization	Anti-COVID-19 drugs
1	57/M	DM/HTN	<i>A. flavus/ A. fumigatus</i> (2)	Bilateral peripheral patchy infiltrates, consolidation	0.272 / <7	<i>Klebsiella pneumoniae, MRSA</i>	ARDS, AKI, MRSA pneumonia, NSTEMI	Amphotericin B	7	Death	Yes	2	HCQ, Azithromycin, Tocilizumab
2	81/M	DM	<i>A. flavus</i> (1)	Bilateral peripheral patchy infiltrates	0.14/31	<i>P. aeruginosa</i>	ARDS, AKI, MODS	Amphotericin B	20	Death	Yes	11	HCQ, Azithromycin, Tocilizumab
3	71/M	HTN, DM,	<i>A. flavus</i> (2)	Bilateral peripheral patchy infiltrates	0.213 /111	<i>S. maltophilia</i>	ARDS, AKI, Septic shock	Voriconazole	17	Cured and discharged	Yes	15	
4	85/F	DM/HTN	<i>A. niger</i> (1)	Bilateral peripheral patchy infiltrates, consolidation	0.136/ <7	MDR <i>Acinetobacter</i> spp.	Septic shock, AKI, NSTEMI, sinusitis, mastoiditis	Voriconazole	13	Death	Yes	8	HCQ, Azithromycin, Tocilizumab
5	51/F	Atrial myxoma,	<i>A. flavus</i> (1)	Bilateral peripheral	0.126/ <7	<i>P. aeruginosa</i>	Acute kidney injury	Voriconazole	10	Cured and Discharged	Yes	0	HCQ, Azithromycin,

		Recent Stroke		patchy infiltrates, consolidation, unilateral pleural effusion									
6	60/M	DM/HTN	<i>A. flavus</i> (I)	Bilateral interstitial infiltrates	0.125/ <7		ARDS, AKI NSTEMI	No antifungal	25	Cured and discharged	No	1	HCQ, Azithromycin, Tocilizumab
7	46/M	DM	<i>A. niger</i> (I)	Bilateral interstitial infiltrates	0.158/ <7	<i>Acinetobacter</i> spp.	Ventilator associated pneumonia	No antifungal	16	Cured and discharged	No	5	HCQ, Azithromycin
8	77/M	DM/HTN	<i>A. fumigatus</i> (I)	Bilateral interstitial infiltrates	0.131 / <7	<i>S. maltophilia</i>	Hospital acquired pneumonia	No antifungal	9	Cured and discharged	No	3	HCQ, Azithromycin, Tocilizumab
9	55/M	DM/ Gas gangrene	<i>A. flavus</i> <i>/A. niger</i> (I)	Unilateral consolidation	0.316/54.7 85	<i>Clostridium perfringens</i> Bacteremia	Septic shock, MODS	No antifungal	7	Death	No	1	HCQ

Accepted Article

Abbreviations: DM: Diabetes Mellitus; HTN: Hypertension; ARDS: Acute respiratory distress syndrome; AKI: Acute kidney injury; MODS: Multi-organ dysfunction syndrome; NSTEMI: Non-ST elevation MI; MDR: Multi-drug resistant; MRSA: methicillin resistant *Staphylococcus aureus*; GMI: Galactomannan index; BG: beta D glucan; HCQ: Hydroxychloroquine

Figure 1. Timelines for patients with severe COVID-19 pneumonia and aspergillosis



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