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COVID-19 and invasive fungal coinfections: A case series at a Brazilian referral hospital

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ABSTRACT
Background: COVID-19 co-infections have been described with different pathogens, including filamentous and yeast fungi.
Methodology: A retrospective case series study conducted from February to December 2020, at a Brazilian university hospital. Data were collected from two hospital surveillance systems: Invasive fungal infection (IFI) surveillance (Mycosis Resistance Program – MIRE) and COVID-19 surveillance. Data from both surveillance systems were cross-checked to identify individuals diagnosed with SARS-CoV-2 (by positive polymerase chain reaction (PCR)) and IFI during hospital stays within the study period.
Results: During the study period, 716 inpatients with COVID-19 and 55 cases of IFI were identified. Fungal co-infection with SARS-CoV-2 was observed in eight (1%) patients: three cases of aspergillosis; four candidemia and one cryptococcosis. The median age of patients was 66 years (IQR 58-71 years; range of 28-77 years) and 62.5% were men. Diagnosis of IFI occurred a median of 11.5 days (IQR 4.5-23 days) after admission and 11 days (IQR 6.5-16 days) after a positive PCR result for SARS-CoV-2. In 75% of cases, IFI was diagnosed in the intensive care unit (ICU). Cases of aspergillosis emerged earlier than those of candidemia: an average of 8.6 and 28.6 days after a positive PCR for SARS-CoV-2, respectively. All the patients with both infections ultimately died.
Conclusion: A low rate of COVID-19 co-infection with IFI was observed, with high mortality. Most cases were diagnosed in ICU patients. Aspergillosis diagnosis is highly complex in this context and requires different criteria.

Introduction

The COVID-19 pandemic has challenged surveillance systems around the world. In addition to COVID-19 per se, co-infections with different pathogens have also been described [1–3]. In this scenario, it is essential to gain a better understanding of aspects related to the host, intensive care unit admission and immunomodulation between SARS-CoV-2 and other microorganisms [4,5].

Bacterial co-infections are more frequently diagnosed than invasive fungal infections (IFI) [1]. IFI, particularly invasive aspergillosis (IA), requires a high level of suspicion from clinicians and robust laboratory investigation [6]. In this report, we describe the clinical presentation, diagnostic methods, therapeutic management and outcome of different invasive fungal infections associated with COVID-19.

Materials and methods

A retrospective case series study was carried out from February to December 2020. Setting: A Brazilian university hospital with 408 beds that operates as a referral facility for COVID-19. Patients with IFI and SARS-CoV-2 were identified via two surveillance systems used at the hospital: IFI surveillance (Mycosis Resistance Program – MIRE) and COVID-19 surveillance.
Active IFI surveillance has been in place at the hospital since 2018, with a special focus on resistance (Mycosis Resistance Program - MIRE). The healthcare team monitor IFIs by actively searching for positive IFI tests (cultures, serological tests, etc.), prescribing antifungals and reporting suspected infections. Cases are identified, compiled and analyzed by a multiprofessional team (infectologists, nurses, biologists, etc.) at weekly meetings aimed at discussing and validating cases of IFI according to the criteria described below.

Case definition of IFI: the following three classifications were used. 1) Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) [7,8]; 2) Clinical Algorithm to Diagnose Invasive Pulmonary Aspergillosis in Critically Ill Patients (AspliCU) [9]; and 3) Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/I-SHAM consensus criteria for research and clinical guidance (CAPA) [10].

With the emergence of SARS-CoV-2 infections in 2020, the hospital began to monitor the number of hospitalized cases of COVID-19 (patients whose clinical condition was indicative of infection or those who exhibited symptoms and were subsequently diagnosed with the disease). Thus, data were compiled on all the patients hospitalized from February to December 2020 who presented with a positive polymerase chain reaction (PCR) result for SARS-CoV-2 in respiratory sampling. Case definition of COVID-19: SARS-CoV-2 PCR detectable in respiratory specimens.

COVID-19 surveillance and MIRE databases were matched to identify patients with IFI and COVID-19 during the study period, which served as the inclusion criterion. The medical charts of the patients were examined to collect demographic, radiological, laboratory, clinical evolution and survival data. Patients already being treated for IFI who were subsequently diagnosed with SARS-CoV-2 were excluded from the study.

The categorical variables are expressed as absolute values and percentages and the continuous variables as mean, median and interquartile ranges (IQRs). The statistical analyses were performed using SPSS Statistics 20 software.

This study was approved by the Research Ethics Committee of the University of Campinas (protocol number 92692418.9.1001.5404 / 3.166.956).

Results

During the study period, 716 inpatients with COVID-19 confirmed by polymerase chain reaction (PCR) and 55 cases of IFI were identified. Eight patients met the inclusion criterion: positive PCR result for SARS-CoV-2 associated with IFI. Of the 716 cases of COVID-19, 44.1% required intensive care during hospitalization. Considering all the hospitalized cases of SARS-CoV-2, 21% ultimately died.

Fungal co-infection was observed in 8 patients (Table 1; Fig. 1). IFI consisted of three cases of aspergillosis, four candidemia and one cryptococcosis. The median age of these patients was 66 years (IQR 58-71 years; range of 28-77 years) and 62.5% were men. Diagnosis of IFI occurred a median of 11.5 days (IQR 4.5-23 days) after admission and 11 days (IQR 6.5-16 days) after a positive PCR result for SARS-CoV-2. A comparison of the diagnostic intervals showed that IFI was diagnosed a median of 11 days (IQR 6.5-16 days) after admission and 11 days (IQR 6.5-16 days) after a positive PCR result for SARS-CoV-2; the negative value (-1) resulted from a patient initially diagnosed with IFI on admission day (D0) of follow-up, who then tested positive (PCR) for COVID-19 on the first day (case 4). The median length of stay (LOS) was 25.5 days (IQR 13.5-40 days; range 13-82 days), not considering the period outside the hospital in case 4.

In most cases, IFI was diagnosed after admission to the ICU (75% of cases). The cases of aspergillosis (three patients) were diagnosed after ICU admission, as follows; an average of 7 days from ICU admission (3, 6 and 12 days after ICU admission), 8 days after implementing mechanical ventilation (3, 9 and 12 days after mechanical ventilation) and 8.6 days after testing positive (PCR) for SARS-CoV-2 (6, 7 and 13 days after PCR for SARS-CoV-2). Galactomannan was not collected from the respiratory specimens of these patients.

Of the four cases of candidemia, one was diagnosed on the admission day of hospitalization and the other three following intensive care treatment. The last three cases were diagnosed later than those of aspergillosis: an average of 18.6 days after ICU admission (13, 14 and 29 days after ICU admission), 18.3 after mechanical ventilation (13, 13 and 29 days after mechanical ventilation) and 28.6 days after receiving a positive PCR result for SARS-CoV-2 (13, 32 and 41 days after PCR for SARS-CoV-2).

In two cases, COVID-19 and IFI occurred simultaneously and outside the ICU: one patient with cryptococcosis (case 8) and the other with endocarditis (Candida orthopsilosis). In the first case, IFI and viral infection symptoms occurred at the same time, although cryptococcosis was diagnosed several days after COVID-19, with the patient exhibiting symptoms of both on admission. The patient diagnosed with endocarditis was hospitalized twice, with a 28-day interval between hospital stays. In this case the diagnosis of IFI occurred one day before that of COVID-19.

Antifungal therapy was used in all cases except two: in the first (case 3), the culture result was interpreted as colonization by the team caring for the patient (subsequently validated as a fungal infection by the IFI surveillance system), and in the second (case 6), the results were only available post mortem.

All eight patients described received corticosteroids; the doses used are presented in Table 1 in terms of prednisone equivalence. Case 5 and 8 were already using immunosuppressive medications (solid organ transplant). No specific SARS-CoV-2 medication was administered. All the patients progressed to intensive care treatment and death (including cases 4 and 8, who required ICU care during hospitalization).

Discussion

In the present study, the rate of fungal coinfection with COVID-19 was 1% among inpatients. Other studies have reported rates of 0.6 [11], 0.7 [3], 1.5 [12] and 5.5% [2]; however, the heterogeneous populations assessed preclude an accurate comparison. In ICU patients with COVID-19, White et al. reported that 26.7% had IFI [13], Bartoletti et al. identified 27.7% with aspergillosis [14] and Salmantón-García et al. observed a cumulative incidence of 1.1-47.4% in patients under mechanical ventilation [15]. Premature ICU admission, respiratory failure and lymphopenia have been reported as risk factors for secondary infections among COVID-19 inpatients [12].

IFI can occur in two different COVID-19 scenarios. The first is in ICU patients with COVID-19, diagnosed with a fungal infection during hospitalization. In this scenario, factors such as mechanical ventilation, central venous access, broad-spectrum antibiotics and the Covid-19 infection itself seem to contribute to the fungal infection [4].

In the second scenario, both infections are diagnosed simultaneously, as described here in two cases. There are reports of this type of simultaneous diagnosis [16]. García-Vidal et al. found that 3.1% of COVID-19 inpatients had a community coinfection, but none had IFI [3]. In our study, there were two cases of simultaneously diagnosed community coinfection with COVID-19 and fungal infection. One patient was a solid organ transplant recipient with cryptococcosis and the other had fungal endocarditis. Both exhibited symptoms of the two infections on admission and the target organ of the fungal infection was not the lung (central nervous system and heart, respectively), suggesting that co-infection was coincidental and had no cause-effect relationship. On the other hand, there are recent
possibly the greatest challenge in cases of aspergillosis is differentiating between colonization and infection. Taccone et al., studied ICU patients and found significantly lower mortality in those considered colonized by Aspergillus spp (38%) when compared to putative (67%) and proven infections (79%) [20]. The new diagnostic criteria proposed appear to be important tools in deciding whether to initiate antifungal treatment.

The reported mortality rate of COVID-19 patients in the ICU without fungal disease is greater than 30% [13]. Based on the CAPA or AspICU criteria, the mortality rate in patients with COVID-19 associated with Aspergillus spp is higher than that recorded in patients without aspergillosis [14]. All the patients in our study evolved to death. Mortality rate varies from 51.1% to 100% in aspergillosis cases and 43.7% to 97.0% for yeast infections [13]. Salmanton-García et al. reported a mortality rate of 52.2% in 186 patients with CAPA [15]. The clinical outcome can be improved with early antifungal therapy [3,13].

Candidemia is less complex to diagnose than aspergillosis and is based on blood culture. In an Italian cohort, a higher rate of candidemia was observed during the COVID-19 pandemic, likely due to the larger number of patients admitted to the ICU, resulting in immunosuppression [21]. Hughes et al. attributed the high number of candidemia cases to complications associated with hospitalization, such as central venous access [11]. The majority of hospital admission during the pandemic are related to COVID-19, resulting in limited beds for other comorbidities [22], overcrowding and failure in preventing healthcare-associated infections (HAI).

The most frequent species reported is Candida albicans [3,11,21,22]. A larger number of Candida non-albicans cases were observed in our study. With respect to the time between the COVID-19 and candidemia diagnoses, in one case (C. orthopsilosis) they occurred simultaneously and three patients presented with candidemia an average of 28.6 days after a positive PCR result for COVID-19 and 18.6 days (average) after ICU admission. White et al. reported candidemia in COVID-19 patients ten days after the positive SARS-CoV-2 PCR diagnosis and nine days after ICU admission; all the patients required mechanical ventilation and a mortality rate of
| Case | Age | Sex | Isolate fungus | Infection | Underlying disease | Systemic steroid / admission kg / day | Antifungal Therapy | EORTC/MSG | NON-Bronchoscopic lavage culture | Follow-up time | Outcome |
|------|-----|-----|----------------|-----------|-------------------|-------------------------------------|-------------------|-----------|---------------------------------|--------------|---------|
| 1    | 16  | F   | Aspergillus    | Pulmonary  | DM / CKD / HYP     | Yes (28)                           | -0.72             | Voriconazole | Probable            | 1            | 12 +    |
| 2    | 37  | M   | Aspergillus    | Pulmonary  | DM / RRT / HYP     | Yes (9)                            | -0.87             | No         | Putative            | -4           | -1 +    |
| 3    | 46  | F   | Candida        | Candidemia | DM / RRT / HYP / OBE| Yes (20)                           | -0.51             | Micafungin | Proven             | 1            | 10 -    |
| 4    | 65  | M   | Candida        | Candidemia | DM / KDT (everolimus)| Yes (1)                           | -0.48             | Fluconazole | Proven             | 32           | 29 -    |
| 5    | 41  | M   | Candida        | Candidemia | DM / RRT / HYP / OBE| Yes (6)                            | -0.98             | No         | Putative            | 41           | 14 +    |
| 6    | 55  | M   | Candida        | Candidemia | DM / HYP / HF / STO| Yes (13)                           | -0.63             | Amphotericin | Proven             | -             | Not applicable |
| 7    | 67  | F   | Candida        | Candidemia | DM / RRT / HYP / OBE| Yes (13)                           | -0.98             | Fluconazole | Proven             | -             | Not applicable |

**Declaration of Interest Statement**

No potential conflict of interest has been reported by the author(s). All authors have reviewed and approved the manuscript. All authors have contributed significantly to the work. The manuscript has not been previously published nor is it being considered for publication elsewhere.

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