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Research Paper

Invasive fungal infections among critically ill adult COVID-19 patients: First experiences from the national centre in Hungary

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ABSTRACT

Introduction: Data suggests that invasive fungal infections (IFI) might complicate COVID-19. Our goal was to describe characteristics of IFI among critically ill COVID-19 adults.
Methods: A retrospective observational case-series analysis was done between March–July 2020. Consecutive patients with critical COVID-19 were eligible, and have been included when proven or putative/probable IFI could be confirmed during their course. For COVID-19 diagnosis, ECDC definitions and WHO severity criteria were followed. Candidaemia was diagnosed according to the ESCMID 2012 guideline. Invasive pulmonary aspergillosis (IPA) was defined following EORTC/MSG, ECMM/ISHAM and modified AspICU criteria. Outcome variables were rates of IFIs, in-hospital all-cause mortality, rate and time to negative respiratory SARS-CoV-2 PCR.
Results: From 90 eligible patients, 20 (22.2%) fulfilled criteria for IFI. Incidence rate for IFI was 2.02 per 100 patient-days at ICU. Patients were mostly elderly males with significant comorbidities, requiring mechanical ventilation because of ARDS. IFI could be classified as candidaemia in 7/20 (40%), putative/probable IPA in 16/20 (80.0%). Isolated species of candidaemia episodes were *Candida albicans* (4/9, 44.4%), *Candida glabrata* (3/9, 33.3%), *Candida parapsilosis* (1/9, 11.1%), *Candida metapsilosis* (1/9, 11.1%). Mold isolates from lower respiratory tract were *Aspergillus fumigatus*, BAL galactomannan positivity was prevalent (16/20, 80.0%). Mortality was 12/20 (60.0%) with a median time to death of 31.0±37.0 (5–89) days. Only 9/20 (45.0%) patients reached SARS-CoV-2 PCR negativity after a median time of 20.0±12.0 (3–38) days.
Conclusion: In this small cohort of critically ill COVID-19 adults, morbidity and mortality related to invasive fungal infections proved to be significant.

Introduction

Coronavirus disease-19 (COVID-19) is a potentially life threatening infection caused by the highly virulent human coronavirus, SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2). In its most severe forms, COVID-19 progresses to multi-systemic dysfunction, including acute respiratory distress syndrome (ARDS) and cytokine storm (CS). Data from the literature suggest that invasive fungal infections (IFI) might also be accounting for additional morbidity in critical COVID-19, especially in patients with the aforementioned complications [1]. Our aim was to assess the burden and characteristics of invasive fungal infections among critically ill adult COVID-19 patients hospitalized at our centre during the first 4 months of the pandemic.

Abbreviations: AMB, amphotericin-B; ANI, anidulafungin; ARDS, acute respiratory distress syndrome; AS, atherosclerosis; BAL, bronchoalveolar lavage; BAR, baricitinib; BDG, beta-D-glucan; BID, twice daily; BSI, bloodstream-infection; BW, body weight; CAS, caspofungin; CHF, chronic heart failure; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease-19; CRP, C-reactive protein; CS, cytokine storm; CT, computed tomography; DEX, dexamethasone; DLBCL, diffuse large B-cell lymphoma; DM, diabetes mellitus; ECDC, European Centre for Disease Prevention and Control; EH, essential hypertension; EUCAST, European Committee on Antimicrobial Susceptibility Testing; ECMM, European Confederation of Medical Mycology; F, female; FLU, fluconazole; FVP, favipiravir; GM, galactomannan; HCQ, hydroxychloroquine; HI, Horowitz index (P_aO_2/F_iO_2); I, intermediate susceptible; IDSA, Infectious Disease Society of America; IFI, invasive fungal infection; IPA, invasive pulmonary aspergillosis; ISA, isavuconazole; ISHAM, International Society for Human and Animal Mycology; ITR, itraconazole; iv., intravenously; IVIG, intravenus immunoglobulin; L-AMB, liposomal amphotericin-B; LDH, lactate dehydrogenase; LPV/r, lopinavir/ritonavir; LOS, lenght of stay; M, male; MIC, minimal inhibitory concentration; MP, methylprednisolone; MYC, micafungin; PCR, polymerase chain reaction; po., orally (*per os*); POS, posaconazole; QD, once daily; R, resistant; S, sensitive; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; TID, three times daily; TOC, tocilizumab; VAP, ventilator-associated pneumonia; VOR, voriconazole

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Methods

Study design

A retrospective, observational case-series analysis of adult patients hospitalized with COVID-19 at our centre between March and July 2020 was carried out. Our centre is the national referral institution of haematological and infectious diseases, and the main COVID-19 center of Hungary. The study was in accordance with the Helsinki Declaration, as well as national ethical standards. The study protocol was approved by the institutional review board.

Patient selection, follow-up

Consecutive patients with severe or critical COVID-19 were eligible for inclusion. Patient identification and selection was done using the hospital database and notes of infectious disease consultations. To overcome selectional bias, all severe or critically ill patients admitted to our intensive care unit (ICU) were assessed for inclusion by the study team, and a separate list containing data of fungal isolates from clinical specimens and fungal biomarker positivities has been generated for cross-checking. Patients were included if they had proven, probable or putative invasive fungal infection(s) during their clinical course with COVID-19 (see below). No *a priori* exclusion criteria were used. Per protocol, in-hospital follow-up was provided for each patient, post-discharge follow-up was not seeked.

Data collection

A database has been established for the study purpose by manual data extraction from hospital records, and anonymized transfer to a standardized case report form. Collected data were: 1) age and gender, 2) comorbidities, 3) length of stay (LOS), 4) clinical and radiological parameters at COVID-19 and IFI diagnosis (symptom onset, oxygen demand, Horowitz index, ARDS and CS, radiomorphology on chest X-ray or computed tomography [CT]), 5) laboratory parameters at COVID-19 and IFI diagnosis (blood leucocyte, absolute neutrophil granulocyte, lymphocyte and platelet counts, hemoglobin, CRP, procalcitonin, serum ferritin, high sensitivity troponin-I, serum interleukin-6, NT-proBNP, serum creatinine, LDH and D-dimer), 6) rate and time to negative respiratory SARS-CoV-2 polymerase chain reaction (PCR), 7) microbiological characteristics (blood, bronchoalveolar lavage [BAL] and mini-BAL cultures, multiplex respiratory PCR panels [adenovirus, coronavirus, human metapneumovirus, human rhinovirus/enterovirus, influenza A and B, parainfluenzavirus, respiratory syncytial virus, A.baumannii, E.cloacae, E.coli, H.influenzae, K.aerogenes, K.oxytoca, K.pneumoniae, M.catarrhalis, Proteus spp., P.aeruginosa, S.marcescens, S.aureus, S.agalactiae, S.pneumoniae, S.pyogenes, C.pneumoniae, L.pneumophila, M.pneumoniae], urinary *Pneumococcus* and *Legionella sp.* antigen tests, serum beta-D-glucan [BDG], galactomannan [GM] antigen from serum and BAL samples), 8) antimicrobial, immunomodulatory therapies and supportive care, 9) study outcomes.

Definitions, diagnostic and therapeutic strategies at our centre during the pandemic

We adhere to the COVID-19 case definitions of *European Centre for Disease Prevention and Control* (ECDC) [2]. A suspected COVID-19 case is confirmed if SARS-CoV-2 nucleic acid is detected by PCR in a clinical specimen. COVID-19 severity is stratified per *World Health Organization* (WHO) criteria [3]. Non-infectious complications of critical COVID-19 disease were defined as ARDS and/or CS. ARDS is identified according to the 2012 Berlin criteria, CS is diagnosed by trends of clinical and laboratory parameters along with the HScore [4, 5]. IFI was defined if presence of a yeast or mold was proven by culture or non-culture based microbiological methods in a clinical sample obtained by a sterile procedure from a physiologically sterile site/fluid, along with a compatible clinical presentation. Candidaemia episodes were diagnosed if *Candida sp.* was recovered from ≥1 blood culture [6]. Cases of invasive pulmonary aspergillosis (IPA) are ascertained following the EORTC/MSG criteria in immunocompromised (proven or probable diagnosis) and modified AspICU criteria in immunocompetent patients (proven or putative diagnosis) [7–9]. In both systems, proven IPA is defined by microscopic analysis (histopathologic, cytopathologic or direct microscopic examination showing hyphae with tissue damage) and/or *Aspergillus sp.* culture recovery from sterile material obtained by bronchoscopic needle aspiration or lung biopsy. For probable IPA diagnosis, EORCT/MSG criteria rely on host factors (see below) with clinical features (typical patterns on chest CT) and mycological evidence (direct testing: microscopy showing fungal elements or *Aspergillus* sp. culture recovery from BAL, bronchial brush or tracheal aspirate; indirect testing: galactomannan antigen or *Aspergillus sp.* PCR from blood, BAL, bronchial brush or tracheal aspirate) of IFI. EORCT/MSG criteria are applied in cases of inherited or acquired severe immunosuppression, such as prolonged neutropenia (eg. active haematological malignancy, allogeneic stem cell transplantation), systemic corticosteroid treatment or T-cell immunosuppressants. Contrarily, other groups of critically ill patients cannot be classified in the absence of host factors. Modified AspICU criteria applicable for this cohort is a clinical algorithm which relies on microbiological evidence of *Aspergillus sp.* from the lower respiratory tract as entry criterion (original AspICU criteria needed positive culture result from tissue or BAL, modified AspICU criteria added serum/BAL galactomannan antigen positivity), and considers clinical (eg. refractory or recrudescent fever, worsening respiratory insufficiency despite antibiotic therapy etc.) and imaging (pulmonary infiltrates by chest X-ray or CT) criteria for putative IPA (facultative host risk factors were also listed in the original AspICU criteria, similar to EORCT/MSG). After study completion, the 2020 ECMM/ISHAM consensus criteria for COVID-19-associated pulmonary aspergillosis have been published. Therefore, cases have retrospectively been re-evaluated according to these criteria as well [10]. Upper or lower respiratory tract colonizations (cases not corresponding to invasive disease) with either *Aspergillus sp.* or *Candida sp.* were not considered for inclusion, per consensus criteria.

At our centre, patients with critical COVID-19 disease are transferred to the ICU from the isolation ward, where on-demand real-time infectious disease consultation is provided to intensivists. To facilitate in-house diagnostic and therapeutic strategies, care of COVID-19 patients was standardised by written protocols and checklists, according to best available literature evidence. From every patient at ICU admission and/or during intubation, 2 sets of blood cultures, a mini-BAL and subsequent BAL for bacterial and fungal cultures and GM, and blood for BDG and GM were obtained on the same day. Mini-BAL is a non-bronchoscopic, blinded lavage technique during which 10-20 mL of saline is inoculated and re-aspirated through the closed breathing circuit of an intubated patient. BAL sampling, along with needle aspiration (if necessary) was performed for infection validation by a pulmonologist expert on a case-by-case basis, if contraindications were not found for bronchoscopy. All patients included in the study received mini-BAL at admission/intubation, and subsequent BAL for verification of pulmonary infection. Serum BDG and GM were tested at admission, and twice weekly thereafter. Positive galactomannan results are confirmed by another clinical sample taken during a separate procedure. Blood cultures, respiratory samples and fungal biomarkers were retaken simultaneously if the patient developed sepsis, had persistent fevers or deteriorated clinically, without other plausible explanations. Sepsis was defined according to SEPSIS-3 criteria, VAP was diagnosed according to the *Infectious Disease Society of America* (IDSA) guidelines [11, 12].

Cultures and non-culture based microbiological diagnostics were executed at the local microbiology laboratory of our centre. Fungal isolation was done on Candida chromagar (CHROMID Candida, bioMérieux, Spain) and Sabouraud agar (Sabouraud Gentamicin Chloramphenicol 2, bioMérieux, Spain). Fungal identification was done by observation of agar culture characteristics, light microscopic morphology and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Vitek-MS V3, bioMérieux, Spain). Yeast and mold susceptibilities for amphotericin-B, azoles (excluding isavuconazole) and echinocandins were tested by broth microdilution (MICRONAUT-AM Antifungal Agents MIC, MERLIN Diagnostika, Germany), isavuconazole susceptibility was tested by E-test (Liofilchem, Italy). Fungal susceptibility interpretations were done following *European Committee on Antimicrobial Susceptibility Testing* (EUCAST) recommendations (www.eucast.org). Beta-D-glucan levels were tested by kinetic turbidimetry, a cut-off value of ≥11 pg/mL corresponded for positivity (*β*-Glucan Test, FUJIFILM Wako Pure Chemical Corporation, Japan). Galactomannan antigen testing was done by enzyme-linked immunosorbent assay, an optical density index of ≥0.5 was interpreted as positivity (Platelia Aspergillus EIA, Bio-Rad, France).

Study outcomes and analysis

Clinical outcome variables were rate and time to in-hospital all-cause mortality, and rates of invasive fungal infections. Virological outcomes were rate and time to negative SARS-CoV-2 respiratory PCR sampling. PCR negativity was defined if ≥2 consecutive respiratory PCR samples taken ≥48 hours were proven to be negative. Incidence regarding IFI was calculated by using cumulative data from hospital records. Continuous variables were expressed as median± interquartile range with minimum-maximum limits, categorical variables were expressed as absolute numbers (n) and percentages (%). Given the expected low incidence of IFIs, modelling of the primary outcome was not planned *a priori*. For reporting, we adhered to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) Statement [13].

Results

Demographic and clinical characteristics

From 90 patients hospitalized with critical COVID-19 at our centre during the study period, 20 (22.2%) patients with a diagnosis of any IFI were identified and included in the study. Incidence was calculated as 2.02 for any IFI episode, 0.91 for candidaemia episodes, and 1.61 for IPA episodes, each per 100 ICU patient-days. Demographic and clinical characteristics are shown in *Table 1*., laboratory and microbiological characteristics are reported in *Table 2*. Patients were from older age cohorts (median age 75.0±13.0 years), and a trend towards male predisposition (11/20, 55.0%) was noted. The median length of hospital stay was 39.0±36.5 (8–135) days. At admittance, 6/20 (30.0%) of patients had a known underlying chronic pulmonary disease, 9/20 (45.0%) had diabetes mellitus, and 5/20 (25.0%) had oncohaematological malignancy. Systemic corticosteroid usage (2/10, 10.0%) and chronic hepatic diseases (1/20, 5.0%) were not prevalent conditions. At COVID-19 diagnosis, nearly all patients required oxygen supplementation (19/20, 95.0%) and most of them had typical radiomorphological features (17/20, 85.0%) of severe SARS-CoV-2 pulmonary infection. Half of the patients had CS, and 65.0% (13/20) had ARDS as complication. At IFI diagnosis, oxygen supplementation rates were mostly unchanged (19/20, 95.0%), and all patients had refractory or recrudescent fever with radiological progression (17/17, 100.0%). Rates of ongoing CS and ARDS were similar (8/20, 40.0% and 13/20, 65.0%, respectively). From ICU admission, median time to candidaemia was 19.5±22.0, while time to IPA diagnosis was 15.0± 11.0 days.

Table 1
Demographic and clinical characteristics of adult patients with critical COVID-19 and invasive fungal infections included in the study.

Parameters	Total (n=20)
Age (years, median±IQR, min–max)	75.0±13.0 (43.0–90.0)
Male gender (n, %)	11 (55.0)
LOS (days, median±IQR, min–max)	7.0±30.3 (43.0–90.0)
ICU LOS (days, median±IQR, min–max)	29.0±24.8 (3.0–88.0)
ICU admittance rate (n, %)	20 (100.0)
Non-immunosuppressive comorbidities (n, %):	
- Essential hypertension	20 (100.0)
- Chronic heart disease	12 (60.0)
- Chronic vascular disease	9 (45.0)
- Chronic pulmonary disease	6 (30.0)
- Chronic renal disease	3 (15.0)
- Chronic hepatic disease	1 (5.0)
- Chronic neurological disease	8 (40.0)
- Diabetes mellitus	9 (45.0)
- Alcohol abuse	2 (10.0)
- Tobacco abuse	6 (30.0)
Immunosuppressive comorbidities (n, %):	
- Ongoing oncological malignancy	4 (20.0)
- Ongoing haematological malignancy	1 (5.0)
- Systemic autoimmune disease	1 (5.0)
- Systemic corticosteroid treatment	2 (10.0)
- Systemic immunosuppressive treatment	0
Clinical characteristics at COVID-19 diagnosis:	
- Horowitz index (median±IQR, min–max)	120±100 (65–300)
- Oxygen supportation demand (n, %)	19 (95.0)
- ARDS (n, %)	13 (65.0)
- Cytokine storm (n, %)	10 (50.0)
- Radiological positivity / all[a]	17 / 20 (85.0)
Clinical characteristics at IFI diagnosis:	
- Horowitz index (median±IQR, min–max)	200±80 (20–250)
- Oxygen supportation demand (n, %)	19 (95.0)
- ARDS (n, %)	13 (65.0)
- Cytokine storm (n, %)	8 (40.0)
- Radiological progression / all[a]	17 / 17 (100.0)

[a] Positive results are given in proportion to all radiological examinations (chest X-rays and thoracic CT scans) performed per patient during ICU stay.

Microbiological characteristics

During ICU stay, 7/20 (35.0%) had at least one episode of documented candidaemia. Among them, 4/20 (20.0%) patients only had candidaemia (from which 1 patient had two consecutive episodes), 2/20 (10.0%) patients had one bacteraemia and one consecutive candidaemia episode, 1/20 (5.0%) patient had two consecutive bacteraemias and two consecutive candidaemias, and 1/20 (5.0%) patient only had bacteraemia. Isolated yeasts were *Candida albicans* (4/7, 50.0%), *Candida glabrata* (3/7, 37.5%), *Candida parapsilosis* (1/7, 12.5%) and *Candida metapsilosis* (1/7, 12.5%). Bacterial bloodstream-infections were caused by *Pseudomonas aeruginosa* (2/4, 50.0%), *Acinetobacter baumannii* (1/4, 25.0%), *Bacillus cereus* (1/4, 25.0%), *Enterococcus faecium* (1/4, 25.0%). Isolates from mini-BAL and BAL samples proved lower respiratory tract infections in 13/20 (65.0%) cases, of which 10/13 (76.9%) were bacterial and 3/13 (23.1%) were probably fungal. Of note, 3/20 (1.5%) patients probably had lower respiratory tract bacterial coinfections. All fungal isolates were *Aspergillus fumigatus.* Among fungal biomarkers, BAL galactomannan positivity was prevalent (16/20, 80.0%), serum GM positivity (8/20, 40.0%) was lower. More than half of patients had a positive serum BDG (11/20, 55.0%). Antifungal susceptibility of isolates recovered from relevant clinical samples are provided in *Supplementary table 1*. *Candida albicans* isolates had typical susceptibility patterns. Fluconazol and voriconazol resistance was detected among one *Candida glabrata* isolate (1/3, 33%), the remaining two isolates were intermediate susceptible (2/3, 66.6%). The *Candida metapsilosis* isolate was echinocandin resistant, the *Candida parapsilosis* isolate had intermediate susceptibility to echinocandins. All *Aspergillus fumigatus* isolates were susceptible to voriconazole, posaconazole, isavuconazole and amphotericin-B.

Table 2
Laboratory and and microbiological characteristics of adult patients with critical COVID-19 and invasive fungal infections included in the study.

Parameters	Total (n=20)
Laboratory characteristics at COVID-19 diagnosis (median±IQR, min−max):	
- White blood cell count (x10^9/L)	8.9±5.5 (3.3−22.0)
- Neutrophil granulocyte count (x10^9/L)	8.0±6.1 (2.7−18.9)
- Lymphocyte count (x10^{9l}/)	0.67±0.23 (0.32−2.63)
- Thrombocyte count (x10^9/L)	201±131 (58−381)
- Hemoglobin level (g/L)	122±19 (70−151)
- C-reactive protein (mg/L)	165±150 (55−355)
- Procalcitonin (μg/L)	0.2±0.6 (0.1−10.0)
- Interleukin-6 (pg/mL)	95.5±143.3 (25.0−5041.0)
- Serum ferritin (μg/L)	1128±921 (258−2843)
- Cardiac Troponin-I (ng/mL)	0.1±0.1 (0.04−1.4)
- NT-proBNP (pg/mL)	9801±13153 (1750−104782)
- Serum cratinine (μmol/L)	102.0±125.5 (36.0−764.0)
- Serum LDH (IU/L)	847.5±488.0 (519.9−1899.0)
- Serum D-dimer (ng/mL)	3820.0±12572.0 (633.0 −122027.0)
Laboratory characteristics at IFI diagnosis (median±IQR, min−max):	
- White blood cell count (x10^9/L)	10.8±5.9 (4.2−34.0)
- Neutrophil granulocyte count (x10^9/L)	8.1±6.0 (1.3−28.4)
- Lymphocyte count (x10^{9l}/)	1.22±0.74 (0.40−4.0)
- Thrombocyte count (x10^9/L)	256±184 (75−450)
- Hemoglobin level (g/L)	93±12 (83−124)
- C-reactive protein (mg/L)	115±143 (1−365)
- Procalcitonin (μg/L)	0.9±1.8 (0.1−19.1)
- Interleukin-6 (pg/mL)	203.0±589.5 (7.0−5647.0)
- Serum ferritin (μg/L)	948±1132 (426−14588)
- Cardiac Troponin-I (ng/mL)	0.1±0.1 (0.01−0.6)
- NT-proBNP (pg/mL)	5716±10823 (189−96423)
- Serum cratinine (μmol/L)	79.0±61.5 (28.0−223.0)
- Serum LDH (IU/L)	553.0±453.0 (200.9−20780.0)
- Serum D-dimer (ng/mL)	3276.5±5340.0 (814.0−96544.0)
Microbiological characteristics[a] (n, %):	
- Any[b] blood culture positivity	8 / 20 (40.0)
- Urinary antigen test positivity	0 / 2
- Respiratory PCR panel positivity	0 / 4
- Any[c] mini-BAL and BAL culture positivity	13 / 19 (73.7)
- Serum galactomannan positivity	8 / 20 (40.0)
- BAL galactomannan positivity	16 / 20 (80.0)
- Serum beta-D-glucan positivity	11 / 20 (55.0)

[a] Positive results are given in proportion to all performed microbiological tests per patient, during ICU stay.
[b] Including bacteraemia and/or candidaemia episodes.
[c] Including bacterial and fungal respiratory pathogens.

Outcomes, diagnostic and therapeutic approaches

Outcomes, diagnostic and therapeutic approaches are shown in *Table 3* and *Table 4*. The final diagnosis was candidaemia in 7/20 (35.0%), and probable / putative IPA in 16/20 (80.0%) patients. All patients in the cohort needed endotracheal intubation and mechanical ventilation, vasopressor therapy and total parenteral nutrition, 18/20 (90.0%) patients were ventilated in prone position. Three patients had positive BAL cultures for *Aspergillus sp.* along with BAL galactomannan assays with high indices. In-hospital all-cause mortality was 12/20 (60.0%) with a median time of 31.0±37.0 (5−89) days to death. Only 9/20 (45.0%) patients reached SARS-CoV-2 PCR negativity with a median time of 20.0±12.0 (3−38) days. Antiviral therapy was chloroquine / hydroxychloroquine (13/20, 65.0%) and favipiravir (5/20, 25.%), while patients with cytokine storm received systemic corticosteroids (9/20, 45.0%), tocilizumab (6/20, 30.0%) and intravenous immunoglobulin (6/20, 30.0%). Empirical antibiotics included piperacillin/tazobactam (14/20, 70.0%) and meropenem (14/20, 70.0%), while empirically administered antifungals were caspofungin (14/20, 70.0%) and amphotericin-B (7/20, 35.0%).

Table 3
Outcomes and therapeutic approaches to adult patients with critical COVID-19 and invasive fungal infections included in the study.

Parameters	Total (n=20)
Final IFI diagnosis[a] (n, %):	
- Episodes of candidaemia	9 (45.0)
- Putative invasive pulmonary aspergillosis	16 (80.0)
In-hospital all-cause mortality (n, %)	12 (60.0)
Time to death from ICU admission (days, median± IQR, min−max)	31.0±37.0 (5.0−89.0)
Rate of SARS-CoV-2 PCR negativity (n, %)	9 (45.0)
Time to SARS-CoV-2 PCR negativity from ICU admission (days, median±IQR, min−max)	20.0±12.0 (3.0−38.0)
Antiviral therapies given (n, %):	
- Chloroquine / hydroxychloroquine	13 (65.0)
- Favipiravir	5 (25.0)
- Lopinavir/ritonavir	3 (15.0)
Antifungal therapies given (n, %):	
- Caspofungin	14 (70.0)
- Fluconazole	4 (20.0)
- Voriconazole	6 (30.0)
- Itraconazole	1 (5.0)
- Isavuconazole	1 (5.0)
- Amphotericin-B	7 (35.0)
Immunomodulatory therapies given (n, %):	
- Tocilizumab	6 (30.0)
- Baricitinib	3 (15.0)
- Intravenous immunoglobulin	6 (30.0)
- Reconvalescent plasma	3 (15.0)
- Systemic corticosteroid	9 (45.0)
Intensive therapies given (n, %):	
- Intubation and mechanical ventilation	20 (100.0)
- Vasopressor therapy	20 (100.0)
- Renal replacement therapy	7 (35.0)
- Total parenteral nutrition	20 (100.0)
- Prone position ventilation	18 (90.0)

[a] two patients had candidaemia and invasive pulmonary aspergillosis coinfections

Discussion

Present study

In this study, conducted among critically ill adult COVID-19 patients hospitalized at a single ICU during a 4-month period, we have calculated that 22.2% of all patients were diagnosed with IFI. Most patients were elderly males, with a relevant comorbidity burden of mostly chronic cardiopulmonary and metabolic diseases. Almost all patients had ARDS and ongoing cytokine storm at diagnosis, prompting for complex intensive care maneuvers and immunomodulatory therapies. Among 20 enrolled cases from 90 eligible patients, 35.0% had at least on episode of candidaemia with 4 distinct *Candida* species. Serum BDG positivity was 55.0%. Probable/putative IPA was diagnosed in 80.0% among 20 included patients. Three patients had BAL fungal cultures positive for *Aspergillus fumigatus*. Serum galactomannan antigen testing had a lower diagnostic yield for IPA (40.0%), while respiratory galactomannan antigen testing was more sensitive (80.0%). Some patients had invasive bacterial coinfections, further complicating adequate antimicrobial strategies. In our cohort, all-cause mortality was high (60.0%) at 31.0±37.0 days.

Previous studies from the literature

According to a review by *Antinori et al.* examining 33 cases with COVID-19 associated IPA from the literature, all patients had been mechanically ventilated at an ICU ward. Patients were predominantly elderly males (81%) with a history of chronic obstructive pulmonary disease (21%) and diabetes mellitus (27%) as comorbidities. The

Table 4

Diagnostic and therapeutic approaches to invasive fungal infections of patients included in the study. ARDS: acute respiratory distress syndrome, AS: atherosclerosis, BAL: bronchoalveolar lavage, BAR: baricitinib (po. 4 mg QD), BDG: beta-D-glucan, BSI: bloodstream-infection, CAS: caspofungin (iv. 70/50 mg once daily), CHF: chronic heart failure, COPD: chronic obstructive pulmonary disease, DEX: dexamethasone (po. 6 mg QD), DLBCL: diffuse large B-cell lymphoma, DM: diabetes mellitus, EH: essential hypertension, F: female, FLU: fluconazole (iv. 12/6 mg/kg BW QD), FVP: favipiravir (po. 1600/600 mg BID), GM: galactomannan, HCQ: hydroxychloroquine (po. 200 mg TID), HI: Horowitz index (P_aO_2/F_iO_2), ICU: intensive care unit, ISA: isavuconazole (po. 200 mg TID for 2 days, then 200 mg QD), ITR: itraconazole (po. 200 mg BID), iv.: intravenously, IVIG: intravenous immunoglobulin (1000 mg/kg BW QD), L-AMB: liposomal amphotericin-B (iv. 3 mg/kg BW QD), LPV/r: lopinavir/ritonavir (po. 500/125 mg BID), LOS: lenght of stay, M: male, MP: methylprednisolone (iv. 40 mg QD), TOC: tocilizumab (iv. 80 mg/kg BW for 2 doses), VAP: ventillator-associated pneumonia, VOR: voriconazole (iv. 6/4 mg/kg BW BID).

Patient	Age (years), gender	Immuno-suppressed state[a]	Medical history[a]	ARDS, cytokine storm[a]	Bacterial co-infection[b]	Candidaemia		Invasive pulmonary aspergillosis (IPA)				Therapy			Outcome, ICU LOS (days)
						Blood culture	*Serum BDG*[c]	*BAL fungal culture*[d]	*BAL GM*[d,e]	*Serum GM*[e]	*Classification of IPA*	*Antifungal treatment*	*Antiviral treatment*	*Immuno-modulatory treatment*	
#1	85, M	No	EH, AS	Moderate (HI 120), no	No	*C. metapsilosis*	Negative	*A. fumigatus*	Positive (3)	Positive (3)	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	FLU and L-AMB	HCQ	MP	Death, 31
#2	79, M	Yes	EH, CHF, AS chronic liver failure, active prostate cancer, alcohol and tobacco abuse	Moderate (HI 200), no	*B.cereus* and *P. aeruginosa* BSI, and *P. aeruginosa* and *S. maltophilia* VAP	*C. albicans* and *C. glabrata*	Positive (487)	Negative	Positive (2)	Positive (1)	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	CAS and FLU, followed by VOR and L-AMB	HCQ	No	Death, 88
#3	76, M	Yes	EH, CHF, active lung cancer	Severe (HI 100), no	*E. faecium* BSI	*C. albicans*	Positive (228)	Negative	Negative	Negative	–	CAS and L-AMB	HCQ LPV/r	IVIG	Death, 28
#4	90, M	No	EH, CHF, vascular dementia	No, no	No	*C. glabrata*	Negative	Negative	Negative	Negative	–	FLU	HCQ	No	Death, 7
#5	77, M	No	EH, CHF, AS, COPD, chronic renal failure, vascular dementia, DM, tobacco abuse	No, no	*K. pneumoniae* VAP	*C. albicans*	Positive (25)	Negative	Negative	Negative	–	CAS	No	No	Death, 24
#6	69, F	No	EH, vascular dementia, obesity, alcohol and tobacco abuse	No, no	No	Negative	Positive (15)	Negative	Positive (3)	Positive (2)	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	ITR	HCQ	TOC	Survival, 18
#7	56, F	No	EH, COPD	Severe (HI 100), yes	*S. maltophilia* VAP	Negative	Positive (94)	Negative	Positive (1.5)	Positive (1)	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	CAS,followed by VOR	FVP	TOC BAR DEX IVIG	Survival, 30
#8	75, M	No	EH	Moderate (HI 120), yes	*P. aeruginosa* VAP	*C. albicans* and *C. parapsilosis*	Positive (82)	Negative	Positive (1.4)	Positive (0.8)	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	CAS, followed by VOR	FVP	TOC BAR DEX IVIG	Survival, 38
#9	90, F	No	EH, CHF, AS, DM	No, no	No	Negative	Positive (113)	Negative	Positive (3)	Positive (1)	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	n.a.	FVP	IVIG	Survival, 8
#10	48, F	Yes	EH, CHF, AS, chronic renal failure, DM, SLE, chronic steroid treatment, hypothyroidism	Moderate (HI 150), yes	*S. maltophilia* and *K. pneumoniae* VAP	Negative	Positive (13)	Negative	Positive (3)	Positive (0.8)	EORCT/MSG: probable AspICU: putative ECMM/ISHAM: probable	CAS	HCQ	TOC	Death, 20
#11	60, F	No	EH, COPD, obesity, tobacco abuse	No, yes	No	Negative	Positive (106)	Negative	Positive (1.5)	Negative	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	n.a.	HCQ	MP Reconvalescent plasma	Survival, 45
#12	43, M	No	EH, obesity	Severe (HI 100), yes	*P. aeruginosa* VAP	Negative	Positive (20)	Negative	Positive (1.2)	Negative	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	CAS and FLU, followed by L-AMB	HCQ LPV/r	DEX IVIG	Survival, 80
#13	70, M	Yes	EH, active colorectal cancer, DLBCL	Moderate (HI 110), yes	*P. aeruginosa* and *S. maltophilia* VAP	Negative	Positive (17)	Negative	Positive (1.3)	Negative	EORCT/MSG: probable AspICU: putative ECMM/ISHAM: probable	CAS, followed by VOR	HCQ LPV/r	DEX IVIG	Survival, 76
#14	77, M	No	EH, CHF, AS, vascular dementia, DM	Severe (HI 100), yes	*S. maltophilia* VAP	Negative	Negative	Negative	Positive (4.3)	Negative	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	CAS, followed by VOR	No	TOC BAR	Death, 15
#15	72, M	No	EH, CHF, AS, COPD, vascular dementia, DM, tobacco abuse	Moderate (HI 150), no	No	Negative	Negative	Negative	Positive (2.9)	Negative	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	CAS, followed by L-AMB	No	Reconvalescent plasma	Survival, 44
#16	75, F	No	EH, CHF, AS, COPD, vascular dementia, DM, hypothyroidism	Severe (HI 100), no	No	Negative	Negative	*A. fumigatus*	Positive (>6)	Positive (5)	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	L-AMB	FVP	DEX	Death, 5
#17	76, F	Yes	EH, CHF, AS, COPD, chronic renal failure, vascular dementia, DM, chronic steroid treatment, tobacco abuse	Moderate (HI 120), no	*P. aeruginosa* BSI and VAP	Negative	Negative	Negative	Positive (1.8)	Negative	EORCT/MSG: probable AspICU: putative ECMM/ISHAM: probable	CAS, followed by L-AMB	HCQ	TOC	Death, 34
#18	60, M	Yes	EH, CHF, DM, active colorectal cancer	No, yes	No	Negative	Negative	*A. fumigatus*	Positive (>6)	Negative	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	CAS and ISA	HCQ	Reconvalescent plasma	Survival, 51
#19	66, F	No	EH, obesity	Severe (HI 100), yes	No	Negative	Negative	Negative	Positive (4.4)	Negative	EORCT/MSG: n.c. AspICU: putative ECMM/ISHAM: probable	CAS, followed by VOR	HCQ	DEX	Death, 17
#20	80, F	No	EH, CHF, DM, vascular dementia	No, yes	*A. baumannii* BSI and *S. aureus* VAP	*C. glabrata*	Negative	Negative	Negative	Negative	–	CAS	HCQ FVP	DEX	Death, 55

n.a. No available data.

n.c. Not classifiable.

[a] Documented at COVID-19 diagnosis among all patients.

[b] During ICU stay among all patients.

[c] BDG values are in pg/mL.

[d] Samples taken during BAL and bronchoscopic needle aspiration among all patients.

[e] GM values are without dimension (optical density index).

authors calculated an overall mortality of 67% among those affected, with *Aspergillus fumigatus* isolated from BAL or tracheal samples as the causative pathogen of IPA in most cases. Serum galactomannan positivity was low (23%), while respiratory galactomannan testing had a better yield (71%). Patients were diagnosed by using the AspICU algorithm [14]. In our cohort, we found a similar rate from serum, but tracheal sampling provided lower positivity compared to results of *Antinori et al.* Therefore, we cannot exclude a higher rate of false negative testing at our centre (eg. due to mucolytic agent use among patients not included in the final cohort).

Incidence and outcomes of IPA among critically ill adult patients with COVID-19 widely vary between centres. In a retrospective chart review done by *Koehler et al.,* putative IPA was diagnosed by AspICU criteria in five of 19 (26.3%) consecutive cases among patients with COVID-19 associated ARDS admitted to the ICU. Voriconazole, isavuconazole and caspofungin were initiated as antifungal therapies. Three patients died. All identified isolates were *Aspergillus fumigatus* [15]. In contrast, *van Arkel et al.* observed an incidence of 19.4% with 3 probable and 3 possible IPA cases using the EORTC/MSG criteria, among a cohort of 31 ICU patients. *Aspergillus fumigatus* could be identified by culturing in five cases as the causative pathogen, mostly voriconazole with anidulafungin was administered. Four patients died (66.7%) [16]. In our cohort, similarly to literature results, all mold isolates recovered from BAL samples were *Aspergillus fumigatus*. Empirical antifungal therapy was mostly amphotericin-B, but targeted deescalation to mostly voriconazole could be performed in some cases. A larger cohort of patients was analysed by *Wang et al.* They found that among 104 patients with COVID-19, 8 (7.7%) had IPA, with risk factors of older age, initial β-lactam/lactamase inhibitor combination therapy, mechanical ventilation and COPD [17].

Data concerning incidence and outcome of candidaemias among critically ill COVID-19 adult patients are scarce. In a review by *Lai et al*. detailing 14 published studies, candidaemia was detected among 4.0% of mostly ICU patients, while a retrospective case-series of 836 hospitalised COVID-19 patients from two UK hospitals found low numbers of candidaemia: only 3 line-releated infections were documented [18, 19]. *Agrifoglio et al.* documented 15 (10.8%) candidaemia cases with *C. albicans, C. parapsilosis and C. glabrata* among 139 critically ill patients. Patients were mechanically ventilated, required vasopressors, had implanted central venous catheters and were receiving total parenteral nutrition and corticosteroids for ARDS at candidaemia diagnosis. The overall calculated mortality was 40% [20]. Moreover, *Antinori et al.* observed a relatively high rate of candidemia (6.9%) among a cohort of 43 COVID-19 patients treated with tocilizumab at the ward or ICU, suggesting that IL-6 blockade might be associated with invasive yeast infections [21]. In our cohort, the rate of candidaemia was higher compared to literature data, possibly reflecting a broader risk factor burden and longer ICU hospitalization times. The choice of empirical antifungal was dominantly caspofungin, targeted fluconazole could be given in some cases.

Limitations

Our study has several limitations. Firstly, this was a case-series analysis done in a single centre. The relatively small sample size and the lack of a matching control group limits exact risk estimation of IFI among COVID-19 patients. Some patients had multiple infectious complications, and a definite cause of death could not be determined, as autopsies of COVID-19 patients were not routinely done during the first wave in Hungary. Some diagnostic uncertainties might have biased our results, eg. the higher rate of serum BDG might partially represent false positivity (eg. due to intravenous immunoglobuline, parenteral nutrition, gauze usage etc.). Exact body weights were not routinely documented at ICU, but presence of obesity based on clinical examination was archived. Finally, it could be challenging to distinguish a colonization from a clinical infection with *Aspergillus sp.* in patients with COPD. Despite these limitations, we think that our study might underscore the importance of clinically relevant, potentially fatal invasive fungal diseases among COVID-19 patients.

Conclusion

In this series of critically ill adult COVID-19 patients, invasive fungal infections including invasive pulmonary aspergillosis and candidaemia seemed to result in relevant morbidity and mortality burden. Further prospective data should be collected on this emerging subject.

Transparency declaration

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Declaration of Competing Interest

The authors declare no conflicts of interest regarding this article. The ICMJE Form for Disclosure of Potential Conflicts of Interest was completed by the corresponding author on behalf of all co-authors.

Ethics approval

The institutional review board approved the study protocol. Approval for the use of *off-label* investigational drugs (tocilizumab, baricitinib, intravenous immunoglobulin) was granted by our institutional review board, as well as the National Institue of Pharmacy and Nutrition in relation to the COVID-19 surge in Hungary (*ogyei.gov.hu/tajekoztato_a_veszelyhelyzet_megszunesevel_kapcsolatos_a_covid_19_-jarvany_idejen_kulonos_meltanylast_erdemlo_betegellatasi_erdekhez_-kotheto_gyogyszeralkalmazasok_bejelenteserol*).

Availability of data

Anonymised data of patients are available from the corresponding author on reasonable request.

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Authors' contributions

BGSZ: management of patients (infectious disease, attending), data collection, data analysis, preparation of the manuscript; BL: management of patients (infectious disease, senior), data collection, preparation of the manuscript; IB: management of patients (intensive care, senior), review of the manuscript; ESZ: microbiological analysis, preparation of the manuscript; JSZ: management of patients (infectious disease, senior), review of the manuscript; VNI: chief of the COVID-19 Centre, preparation of study protocol, preparation and review of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.mycmed.2021.101198.

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