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Invasive cryptococcal disease in COVID-19: systematic review of the literature and analysis

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SUMMARY

During the Coronavirus Disease 2019 (COVID-19) pandemic, an increasing number of fungal infections associated with SARS-CoV-2 infection have been reported. Among them, cryptococcosis could be a life-threatening disease. We performed a Systematic Review (PRISMA Statement) of cryptococcosis and COVID-19 co-infection, case report/series were included: a total of 34 cases were found, then we added our case report. We collected patients' data and performed a statistical analysis comparing two groups of patients sorted by outcome: "dead" and "alive". Three cases were excluded for lack of information.

To compare categorical data, we used a Fisher-exact test (α=0.05). To compare quantitative variables a U Mann-Whitney test was used (α=0.05), with a 95% Confidence Interval.

A total of 32 co-infected patients were included in the statistical analysis. Mortality rate was 17/32 (53.1%): these patients were included in "dead" group, and 15/32 (46.9%) patients survived and were included in "alive" group. Overall, males were 25/32 (78.1%), the median age was 60 years (IQR 53-70) with non-statistically significant difference between groups (p=0.149 and p=0.911, respectively).

Three variables were associated with mortality: ARDS, ICU admission and inadequate treatment. Overall, 21 out of 24 (87.5%) patients were in ARDS with a statistically significant difference among two groups (p=0.028). ICU admission for COVID-19 was observed in 18/26 (69.2%), more frequently among dead group (p=0.034). Finally, 15/32 (46.9%) patients had adequate treatment (amphotericin B + flucytosine for invasive cryptococcosis) mostly among alive patients (p=0.039). In conclusion, mortality due to cryptococcal infection among COVID-19 patients remains high but an early diagnosis and appropriate treatment could reduce mortality.

Keywords: Cryptococcosis, systematic review, COVID-19, SARS-CoV-2, fungal infection.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) pandemic, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), nowadays represents the major health problem world-

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wide. With the increase of severe COVID-19 cases, several opportunistic infection complications emerged, including bacterial and fungal infections. Indeed, fungal infections have been reported among patients with COVID-19 infection [1-4]. Among fungal infections, cryptococcosis is relatively common in immunosuppressed patients: about 80% of cases are associated with Human Immunodeficiency Virus (HIV) infection [3-6]. The incidence is estimated from 0.04% to 12% per year among HIV patients [7, 8].

The genus *Cryptococcus* comprises above 20 species that usually do not reside as part of the human microbiota, though endobronchial colonization was seen in patients with chronic obstructive pulmonary disease (COPD) [6]. Generally, only 2 species - *Cryptococcus neoformans* (*C. neoformans*) and *Cryptococcus gattii* (*C. gattii*) - affect humans and animals. These fungi are commonly found around the world in soil; the lung is considered the entrance door in humans and the sites of infection are the pulmonary system and Central Nervous System (CNS), but other sites can also be affected. Among patients with low CD4+ count or in treatment with immunomodulant agents, *i.e.* steroids or other immunomodulant therapy, the most common manifestations of cryptococcal infection include pneumonia and central nervous involvement, mainly meningitis [9]. Moreover, other underlying conditions, such as long-term treatment with steroids, solid organ transplantation (SOT), malignancies, HIV, type II diabetes mellitus (DM) are considered risk factors for the development of cryptococcal infection [6].

Patients with COVID-19, particularly with severe forms, can develop lymphopenia with decreased CD4+ and CD8+ T lymphocytes count, associated with alteration in cytokine levels (IL-2, IL-6, IL-10, IFN-y) [10]. This can increase the susceptibility to fungal infections, including cryptococcosis [8-10]. The observation of a case of a 70-year-old man (without any known risk factors) who died with COVID-19 and cryptococcosis co-infection gave us the opportunity to focus attention on this potentially fatal disease.

MATERIAL AND METHODS

PROSPERO reference

The review is available online on PROSPERO, CRD42022323114.

Design of study

Two independent researchers (G.P. and D.S.) performed a Systematic Review of the literature of SARS-CoV-2 and cryptococcal co-infections via the databases PubMed and Embase, according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [11].

All references listed were hand-searched for other relevant articles. PRISMA algorithm is shown in Figure 1.

Study population

We included articles or abstracts, without any restrictions, to find papers describing cases in which patients with SARS-CoV-2 infection and cryptococcosis were reported. The research period was 01/01/2020 to 18/03/2022. The searched terms for both databases were ["cryptococc* AND (COVID-19 OR SARS-CoV-2)"], ["cryptococcosis AND SARS-CoV-2"], ["cryptococcosis AND COVID-19"], ["cryptococcemia AND SARS-CoV-2"], ["cryptococcemia AND COVID-19"], ["cryptococcus AND SARS-CoV-2"], and ["cryptococcus AND COVID-19].

Definitions

Cryptococcal infection was defined "pre-COVID-19" if signs/symptoms and/or microbiological sample and/or radiological examination compatible with cryptococcosis were present "before" a positive nasal swab and/or signs/symptom compatible with SARS-CoV-2 infection; cryptococcal infection was defined "post-COVID-19" if signs/symptoms and/or microbiological sample and/or radiological examination compatible with cryptococcosis were present "after" a negative nasal swab for SARS-CoV-2. We used WHO definition of Severe COVID-19. Immunomodulator was defined as a drug that can vary the activity of the immune system.

Aim

The aim of our review was to evaluate any mortality risk factors associated with cryptococcal and SARS-CoV-2 co-infection.

Statistical analysis

Version 27.0.1.0 of IBM® SPSS® Statistics was used for statistical analysis by G.P.

All the manuscripts found in the systematic review (including our case report) were included in

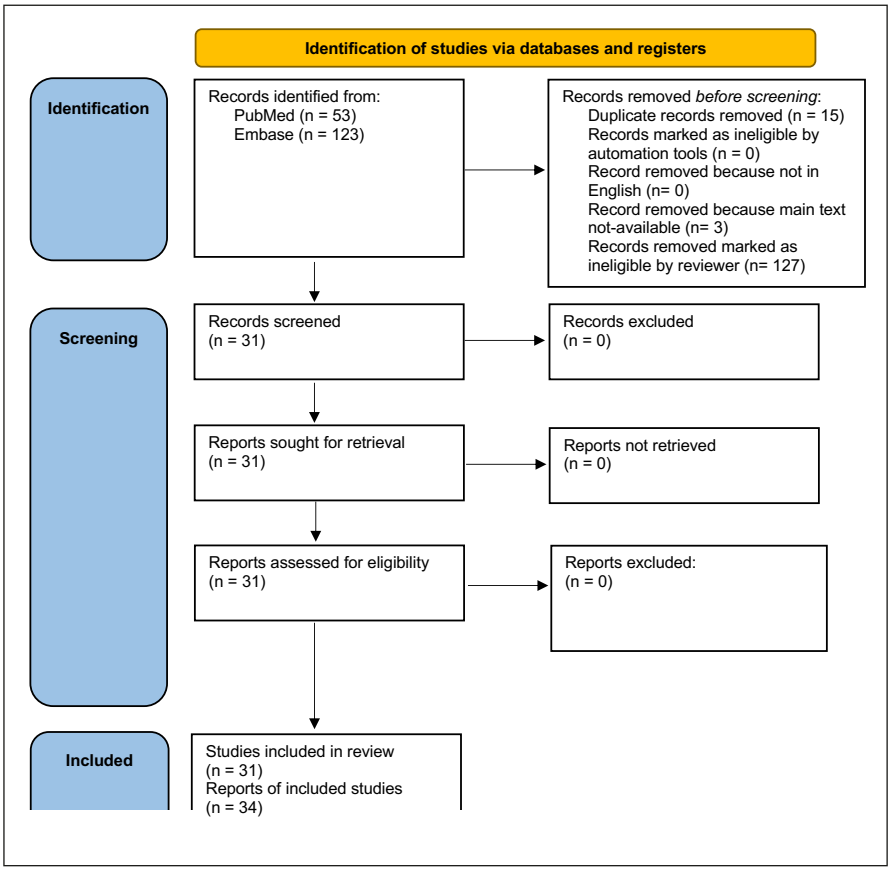

Figure 1 - Article selection according to PRISMA criteria [11]. Number of studies included: 31. One manuscript is a case series (4 patients), total reports included.

the statistical analysis. Cases without relevant information such as sex, age, treatment or outcome were excluded.

Quantitative variables are shown as median and InterQuartile Range (IQR) 25%-75%.

We also evaluated the association between admission in Intensive Care Unit (ICU) due to a severe COVID-19 and mortality: we excluded cases with no available clarification about patients' admission in ICU; we also excluded cases in which patients were admitted in ICU for cryptococcal infection and not for a severe COVID-19.

The only combination of amphotericin B plus flucytosine was defined as "Adequate treatment" in case of invasive cryptococcosis and in case of severe pulmonary cryptococcosis according to literature [12-15].

Clinical characteristics of all the cases retrieved are analytically shown in Supplementary table 1. Statistical analysis is shown in Supplementary table 2. Patients were divided into two groups by outcome: dead and alive patients. A one-sided Fisher-exact test was used to compare categorical data (qualitative variables), α=0.05. A U Mann-Whitney was used to compare quantitative variables (Age, Time from SARS-CoV-2 infection to Cryptococcal infection, Lymphocyte nadir, CD4+ nadir), α=0.05, with a 95% Confidence Interval.

RESULTS

One hundred seventy-six articles were found in our review: 145 articles were removed before screening, because no cryptococcal case was found; we included the remaining 31 articles with a total of 34 case reports [16-46].

In Supplementary Table 1 all the patients' demographic and clinical characteristics were collected and listed.-Three articles were excluded in the statistical analysis, because relevant information (such as sex, age, comorbidities, treatment, and outcome) were not available in the main text [16,

38, 45]. A total of 32 case reports were analyzed. Data are shown in Supplementary Table 2.
Most of patients were males 25/32 (78.1%), the median age was 60 years (IQR 53-70) with non-statistically significant difference between two groups (dead and alive) [respectively, p=0.149 and p=0.911]. Overall, 17/32 (53.1%) of enrolled patients died and 15/32 (46.9%) survived.
The median lymphocytes count was 400 cells/μL (IQR 400-700), and the median CD4+ values was 252 cells/μL (IQR 70-438), with no statistically significant differences between the alive and dead group (p=1 and p=0.190, respectively).
Overall, 28/32 (87.5%) of total had at least one co-morbidity without significant difference among groups (p=0.319).
The most frequent found co-morbidities were arterial hypertension (53.1%), type II DM (43.8%), SOT (21.9%) and obesity (21.9%), neither of them was associated with mortality (p=0.356, p=0.252, p=0.424 and p=0.648 respectively).
Data on HIV infection was available only for 22 patients, 3 of which were HIV positive (13.6%) with non-statistically significant difference among dead and alive patients (p=0.642); cirrhotic patients were 3/32 (9.4%) without statistically significant difference between groups (p=0.576).
Data about steroid prescription were found only in 28 subjects, the 82.1% (23/28) of them took steroids without statistically significant difference among groups (p=0.428).
The median time from admission in hospital to cryptococcal diagnosis was of 22 days (IQR 13-40), without significant differences between patients who survived and those who died (p=0.727).
Most common site of infection was the bloodstream (23/25 cases) lumbar puncture (LP) was positive for *Cryptococcus* in 17/21 (81%) of total cases, LP wasn't performed by clinician mainly due to clinical instability. Evidence of pulmonary cryptococcosis was found in 7/7 patients' microbiological examination of pulmonary specimen - bronchoalveolar lavage (BAL) in all cases except for a tracheal aspirate.
Among the 4 patients younger than 40-year-old, only one survived and 3/4 died: a 28-year-old male with a previous kidney SOT; a 24-year-old male patient with hemolytic anemia complicated with Pulmonary Thrombo-Embolism and necrotic encephalitis; finally, a 38-year-old and 24-year-old male HIV patients.
Acute Respiratory Distress Syndrome (ARDS), ICU admission and inadequate treatment were associated with death.
Data on ARDS was available for 24 patients: 21 out of 24 (87.5%) developed ARDS with a statistically significant difference among two groups (p=0.028).
ICU admission for COVID-19 was observed in 18/26 (69.2%) of patients - data not available for 6 patients - most of them died (p=0.034).
Overall, 15/32 (46.9%) patients had adequate treatment, alive patients more frequently were treated appropriately 10/15 (66.7%) despite dead patients 5/17 (29.4%) with a significant difference (p=0.039). In 2/32 (6.3%) cases the antifungal treatment was not administered because the diagnosis was post-mortem.

DISCUSSION

Analysis of data shown that cryptococcosis during severe COVID-19 is a rare but life-threatening complication. Most of cryptococcosis cases in our review occurred in male, as reported in literature [6]. Moreover, 80% of non-COVID-19 patients with cryptococcosis had at least one co-morbidity, this is in line with our review: 87.5% of patients carried at least one co-morbidity [9, 10]. The immunological dysregulation during COVID-19 may play a role in a worse outcome of cryptococcosis; indeed, cryptococcal infections are associated with immunodepression, as seen in cases of HIV before the introduction of Highly Active Antiretroviral Therapy (HAART), or other diseases with iatrogenic immunological impairment, such as transplant [3, 6-10]. A recent review of the death due to cryptococcal infection in Brazil - decade 2000-2012 - shows that 75% of cryptococcal cases was due to HIV and 5.5% was associated with other immunodeficiency conditions [8]. In our review, 12/28 (42.9%) patients had at least one classical risk factor for immune-impairment (SOT, HIV, cirrhosis), and in 23/28 (82.1%) patients a steroid was used - in most of cases due to COVID-19 pneumonia.
Moreover, our patients presented lymphopenia, with low CD4+ count, probably due to SARS-CoV-2 infection.
In our study we found an association between admission in ICU, ARDS and inadequate cryptococcal treatment and mortality.

Our study shows a 53.1% mortality, that is slightly higher than reported in literature (range 10%-25%) [6, 12-14, 47, 48]. To note that in some HIV population, a 100% mortality was found for invasive cryptococcosis without antifungal therapy [15]. The higher mortality can be explained by the presence of multiple mortality risk factors such as age, severe COVID-19, ARDS, ICU admission, inadequate treatment. The high mortality in cryptococcal and COVID-19 co-infection could be justified by other usual risk factors associated with mortality as lymphopenia, steroid use, hyperinflammation due to both infections. Our analysis showed no statistical difference for lymphopenia, steroid use or hyperinflammatory state, maybe due to the small number of patients, only case report or case series were found, and a publication bias.

It is be interesting to note that only 8 among the 17 dead patients underwent an LP and 7/8 (87.5%) of LP resulted positive for *Cryptococcus* spp.

Cerebral spinal fluid (CSF) analysis was not performed in 9/17 dead patients, of which 8/9 were not treated with amphotericin B plus flucytosine, standard treatment for invasive cryptococcosis.

We don't know if a LP had been performed on the 8 dead patients not treated with amphotericin B+ flucytosine, Cryptococcus would have been found in CSF.

Some authors investigated the role of cryptococcal protease during SARS-CoV-2 infection, suggesting the possible role of protease, ureases, phospholipases released by Cryptococcus in activating the SARS-CoV-2 S protein leading to hyper-inflammatory response [49]. This could explain the high mortality we found in our review.

In conclusion, lymphopenia and low CD4+ cells levels could lead to cryptococcal infection also in non-HIV patients as well as transplant recipients [51]. Lymphocyte T helper dysregulation due to drugs or disease could represent the pathophysiological base for cryptococcal disease in both transplant recipients and COVID-19 patients [10, 50-53]. Invasive cryptococcosis can be a life-threating disease and an early diagnosis and appropriate treatment could help clinicians to reduce mortality.

Conflict of interests
None to declare.

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Availability of data and materials
Data are available on appropriate request to the corresponding author.

Consent for publication
All authors give their consent for publication.

Authors' contributions
GP, DS, AS, FO, FDL, AF, CB and CI were involved in the patients care; GP, DS, NP, AC and CI were involved in the study design; GP wrote the initial draft of the manuscript; GP and DS performed systematic review; GP performed statistical analysis; NP and AC were involved in development and methodology; NP, AC and CI critically review the manuscript; all authors have read and agree to the published version of the manuscript.

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Supplemental Table 1 - Population characteristics are shown.

	Country	*Sex*	*Age (years)*	*Co-morbidity*	*Immunomodulator Drugs*	*HIV*	*ARDS*	*ICU*	*Days from admission*	*COVID-19 relationship*	*Treatment*	*Outcome*	*EORTC-MSG criteria*	*Site of infection*	*Radiologic features*	*Cryptococcus spp.*	*Lymphocite (cells/ μL)*	*CD4+ (cells/ μL)*
[16]	China	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Concurrent	n.a.	n.a.	n.a.	Blood (culture)	n.a.	Cryptococcus spp.	n.a.	n.a.
[17]	Perù	M	38	HIV	No	Yes	Yes	n.a.	1	Pre-COVID	Amb+ fluconazole	Dead	Proven	CNS (Ag)	Chest CT: GGO, bilateral Infiltrates. Brain MRI: Hydrocephalus, T1 hypo-intense nodules in IV Ventricular	C. neoformans	"Low"	438
[18]	Brazil	M	75	AH, SOT (KT), Liver Cirrhosis	Yes (steroid, FK)	No	Yes	Yes	12	Concurrent	Fluconazole	Dead	Proven	Blood (culture)	GGO	C. neoformans	114	n.a.
[19]	Qatar	M	60	AH, DM, CVD	Yes (TCZ, steroid)	No	Yes	Yes	> 48	Concurrent	Amb+flucy	Dead	Proven	Blood (culture)	n.a.	C. neoformans	n.a.	n.a.
[20]	U.S.A.	F	73	No	Yes (steroid)	No	No	No	> 17	Concurrent	L-Amb+flucy	Alive	Proven	CNS (culture)	Brain CT: Hydrocephalus (CT)	C. neoformans	700	n.a.
[21]	Canada	M	24	Haemolitic Anemia	Yes (steroid)	No	Yes	Yes	20	Concurrent	None	Dead	Proven	Blood (culture)	Chest CT: TEP, GGO. Brain MRI: Temporal lobes, basal ganglia, thalami necrosis	C. neoformans	n.a.	n.a.
[22]	U.S.A.	M	75	DM, AH, obesity, osteoarthritis	Yes (steroid)	No	Yes	Yes	26	Concurrent	None	Dead	Proven	Blood (culture)	n.a.	C. neoformans	400	n.a.
[23]	Spain	M	78	DM, AH, CKD (sIV)	Yes (steroid)	No	Yes	Yes	75	Post-COVID	Amb+flucy	Alive	Proven	Blood and CNS (Ag, culture)	Chest CT: Cavitated lesions (LUL)	C. neoformans	200	n.a.
[24]	Brazil	M	28	SOT (KT)	Yes (FK+MF, steroid)	n.a.	n.a.	Yes	10	Concurrent	Amb	Dead	Proven	CNS (culture)	n.a.	n.a.	n.a.	n.a.
[25]	U.S.A.	M	24	HIV	No	Yes	No	Yes*	1	Concurrent	Amb+flucy	Alive	Proven	CNS (culture)	Brain MRI: T2 hyper-intense foci in MRI (also cystic in caudate and putamina; subcortical & periventricular white matter of the frontal lobe)	C. neoformans	980	"Low"
[26]	U.S.A.	M	78	AH, COPD	Yes (steroids)	No	Yes	Yes	22	Post-COVID	L-amb	Dead	Proven	Lung (culture)	n.a.	C. neoformans	400	70
[27]	U.S.A.	M	52	DM, alcoholism	No	No	n.a.	n.a.	n.a.	Concurrent	L-amb + flucy.	Alive	Proven	n.a.	Chest CT: GGO; Brain CT: Periventricular, subcortical attenuation (CT)	C. neof./gattii	"Low"	"Low"
[28]	U.S.A.	F	78	AH, DM	No	No	Yes	Yes	n.a.	Concurrent	fluconazole	Dead	Probable	Lung (culture)	n.a.	n.a.	n.a.	n.a.
[29]	U.S.A.	M	55	LTBI	No	No	No	Yes*	21	Post-COVID	L-amb+flucy	Alive	Proven	Lung, blood, CNS (culture, Ag)	n.a.	C. gattii	n.a.	339
[30]	U.S.A.	M	66	Liver cirrhosis, obesity, DM	Yes (steroids)	No	Yes	Yes	22	Concurrent	Amb+flucy.	Dead	Proven	Blood and CNS (culture)	Chest CT: Bilateral infiltrate	C. neoformans	n.a.	n.a.
[31]	U.S.A.	F	76	AH, GERD	Yes (TCZ, steroids)	No	Yes	Yes	44	Post-COVID	Amb+flucy	Alive	Proven	Blood and CNS (culture)	Brain MRI: Multiple cerebellar and cerebral infarct	C. neoformans	400	n.a.
[32]	U.S.A.	F	70	AH, obesity, SOT (KT)	Yes (AZA, FK, steroids)	n.a.	n.a.	No	1	Concurrent	Fluconazole	Alive	Proven	Blood and CNS (Ag, culture)	n.a.	n.a.	n.a.	n.a.
		M	63	AH, DM, SOT (KT)	Yes (MF, FK, steroids)	n.a.	n.a.	No	20	Concurrent	Amb+flucy	Alive	Proven	Blood and CNS (Ag, culture)	n.a.	n.a.	n.a.	n.a.
		M	62	AH, DM, SOT (HT)	Yes (FK, SIR)	n.a.	n.a.	No	93	Concurrent	Amb+flucy	Alive	Proven	Blood and CNS (Ag, culture)	n.a.	n.a.	n.a.	n.a.
		F	53	AH, DM, SOT (KT)	Yes (MF, FK, steroids)	n.a.	n.a.	No	95	Concurrent	Amb+flucy	Alive	Proven	Blood and CNS (Ag, culture)	n.a.	n.a.	n.a.	n.a.
[33]	U.S.A.	M	70	Ictus cerebri, CVD, AH, COPD, MRGE, CKD, obesity	Yes (steroids)	n.a.	Yes	Yes	34	Concurrent	Micafungin	Dead	Proven	Blood (culture)	Chest CT: Bilateral GGO	C. neoformans	510	n.a.
[34]	U.S.A.	M	57	AH	Yes (steroids)	No	Yes	Yes	36	Concurrent	L-Amb+flucy	Dead	Proven	Blood and CNS (culture)	Chest CT: Bilateral infiltrates	C. neoformans	n.a.	n.a.
[35]	U.S.A.	M	59	COPD, CVD, Obesity, DM, Cirrhosis, HF, AH	Yes (steroids, cyclophosphamide)	n.a.	Yes	Yes	33	Concurrent	L-Amb+flucy	Dead	Proven	Lung (culture)	Chest CT: Bilateral GGO	C. neoformans	n.a.	n.a.
[36]	U.S.A.	M	59	AH, DM, obesity	Yes (steroids)	No	Yes	Yes	10	Concurrent	L-amb	Alive	Proven	Blood (culture)	Chest CT: Bilateral GGO	C. neoformans	n.a.	636

	Country	*Sex*	*Age (years)*	*Comorbidity*	*Immunomodulator drugs*	*HIV*	*ARDS*	*ICU*	*Days from admission*	*COVID-19 relationship*	*Treatment*	*Outcome*	*EORTC-MSG criteria*	*Site of infection*	*Radiologic features*	*Cryptococcus spp.*	*Lymphocite (cells/ μL)*	*CD4+ (cells/ μL)*
[37]	Brazil	M	70	SOT (liver)	Yes (MF, steroids)	No	Yes	Yes	34	Concurrent	No	Dead	Proven	Blood (culture)	n.a.	*C. neoformans*	n.a.	n.a.
[38]	Mexico (3 patients)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
[39]	Uganda	F	37	HIV, active tuberculosis	Yes (steroids)	Yes	Yes	No	n.a.	Concurrent	Fluconazole	Dead	Proven	Blood and CNS (Ag)	Chest Rx: Reticolonodular and GGO; Brain CT: hypodensity of fronto-parieto right cortex	n.a.	530	32
[40]	Brazil	F	54	Obesity, DM, CVD, AI	n.a.	n.a.	Yes	Yes	n.a.	Concurrent	Amb+ fluconazole	Alive	Proven	Blood (culture)	n.a.	*C. laurentii*	n.a.	n.a.
[41]	Germany	M	55	AF, dilatated cardiomypathy	Yes (ssteroids)	No	Yes	Yes	14	Concurrent	Amb+flucy	Alive	Probable	Lung (culture)	Chest CT: GGO	*C. neoformans*	400	n.a.
[42]	U.S.A.	M	62	No	Yes (steroids)	No	Yes	n.a.	n.a.	Post-COVID-19	Fluconazole	Alive	Proven	Blood (Ag)	n.a.	n.a.	n.a.	n.a.
[43]	India	M	60	AH, DM, primary hypotiroidism	Yes (steroids)	n.a.	n.a.	No	n.a. (>60)	Post-COVID-19	L-Amb	Alive	Proven	Lung (biopsy)	Chest CT: air space consolidation, surrounding ill-defined ground glassing with interlobular septal thickening	*C. neoformans*	n.a.	n.a.
[44]	Brazil	M	49	No	n.a.	No	Yes	n.a.	n.a.	Pre-COVID-19	Amb	Dead	Proven	Blood and CNS (culture)	n.a.	*C. neoformans*	"Low"	n.a.
[45]	U.S.A.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
[46]	U.S.A.	M	56	DM, gout	Yes (steroids)	No	Yes	Yes	29	Concurrent	L-Amb+flucy	Dead	Proven	Blood and CNS (Ag, culture)	n.a.	*C. neoformans*	n.a.	71
Our Case Report	Italy	M	70	PE, asthma	No	No	Yes	Yes	57	Concurrent	Amb	Dead	Proven	Lung and blood (Ag)	Chest CT: GGO, cavitated lesions	*C. neoformans*	720	252

Abbreviations: M = male, F = female. DM = Type II diabetes mellitus. AH = arterial hypertension. CKD = chronic kidney disease. s (IV) = stage IV. AF: atrial fibrillation. CVD = cardiovascular disease. GERD = Gastroesophageal reflux disease. COPD = Chronic Obstructive Pulmonary Disease. SOT = solid organ transplantation. KT = kidney transplantation. COVID-19: Coronavirus Disease 2019. ARDS: Acute Respiratory Distress Syndrome. ICU = intensive care unit. HIV: Human Immunodeficiency Virus. Ag: cryptococcal antigen. PCR: polymerase chain reaction. Amb = amphotericin B. L-Amb = liposomal Amphotericin B. Flucy = flucytosine. EORTC-MSG: European Organization for Research and Treatment of Cancer Mycoses study group. CNS: Central Nervous System. Ag = antigen. GGO = ground glass opacities. TEP = pulmonary thrombo-embolism. LUL = Left Upper Lobe. CT = computed tomography. MRI = magnetic resonance imaging. Rx = radiography. Immunomodulators drugs were considered: Steroids. FK: Tacrolimus. MF: mycophenolate. AZA: Azathioprine. SIR: Sirolimus. TCZ = tocilizumab. Days from admission = days from admission to cryptococcal diagnosis. n.a. = not available. COVID-relationship = temporal relationship between COVID-19 and cryptococcosis
Note: *= ICU admission for cryptococcosis.

Supplementary Table 2 - Risk factors associated with death in patients with SARS-CoV-2 infection and cryptococcosis.

	Overall (n= 32)	*Alive (n= 15)*	*Dead (n= 17)*	*p-value*
Male sex – n (%)	25/32 (78.1%)	10/15 (66.7%)	15/17 (88.2%)	0.149
Age (years) – median (IQR 25-75)	60 (53-70)	60 (54-70)	60 (43.5-72.5)	0.911
At least one comorbidity – n (%)	28/32 (87.5%)	12/15 (80%)	16/17 (94.1%)	0.319
Arterial hypertension	17/32 (53.1%)	9/15 (60%)	8/17 (47.1%)	0.356
Type II diabetes mellitus	14/32 (43.8%)	8/15 (53.3%)	6/17 (35.3%)	0.252
SOT	7/32 (21.9%)	4/15 (26.7%)	3/17 (17.7%)	0.424
Obesity	7/32 (21.9%)	3/15 (20%)	4/17 (23.4%)	0.648
Liver Chirrosis	3/32 (9.4%)	0/15 (0%)	3/17 (17.7%)	0.576
None	4/32 (12.5%)	3/15 (20%)	1/17 (5.9%)	0.253
HIV positive	3/22 (13.6%)	1/9 (11.1%)	2/13 (15.4%)	0.642
Immunodeficiency (SOT, HIV, cirrhosis)	12/28 (42.9%)	5/13 (38.5%)	7/15 (46.7%)	0.479
Use of steroids	23/28 (82.1%)	10/13 (76.9%)	13/15 (86.7%)	0.428
ARDS related to COVID-19	21/24 (87.5%)	5/8 (62.5%)	16/16 (100%)	0.028
ICU admission for COVID-19	18/26 (69.2%)	5/11 (45.5%)	13/15 (86.7%)	0.034
Days from admission to diagnosis – median (IQR 25-75)	22 (13-40)	20 (10-75)	27.5 (18-34.5)	0.727
Adequate treatment – n (%)	15/32 (46.9%)	10/15 (66.7%)	5/17 (29.4%)	0.039
Site of infection - Central Nervous System	17/21 (80.9%)	10/13 (76.9%)	7/8 (87.5%)	0.502
Blood stream infection	23/25 (92%)	10/11 (90.9%)	13/14 (92.9%)	0.697
Pulmonary cryptococcosis	7/7 (100%)	3/3 (100%)	4/4 (100%)	1
Lymphocyte (cells/ µL) – median (IQR 25-75)	400 (400-700)	400 (300-840)	455 (328.5-577.5)	1
Lymphocyte T CD4+ (cells/ µL)	252 (70-438)	487.5 (339 -)	71 (51- 345)	0.190

Data from the literature plus our case report: a total of 35 cases were found, 3 patients were excluded due to missing relevant information. Contingency table for categorical variables (Fisher-exact test, a=0.05, one tailed), U Mann-Whitney for numerical variables (a=0.05).
Abbreviations: IQR: Interquartile range. HIV: Human Immunodeficiency Virus. SOT = Solid Organ Transplant. COVID-19: Coronavirus Disease 2019. ARDS: Acute Respiratory Distress Syndrome. EORTC-MSG: European Organization for Research and Treatment of Cancer Mycoses study group. CNS: Central Nervous System.