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## Mycotic infection prevalence among patients undergoing bronchoalveolar lavage with search of SARS-CoV-2 after two negative nasopharyngeal swabs

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## PAPER

# Mycotic infection prevalence among patients undergoing bronchoalveolar lavage with search of SARS-CoV-2 after two negative nasopharyngeal swabs

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E-mail: [filippo.patrucco@gmail.com](mailto:filippo.patrucco@gmail.com)**Keywords:** COVID-19, SARS-CoV-2, *Aspergillus*, *Candida*, *Pneumocystis*, bronchoalveolar lavage

## Abstract

The evidence that severe coronavirus disease 2019 (COVID-19) is a risk factor for development of mycotic respiratory infection with an increased mortality is rising. Immunosuppressed are among the most susceptible patients and *Aspergillus* species is the most feared superinfection. In this study we evaluated mycotic isolation prevalence on bronchoalveolar lavage (BAL) of patients who underwent bronchoscopy in search of severe acute respiratory coronavirus 2 (SARS-CoV-2) RNA. Moreover, we described the clinical characteristics and main outcomes of these patients. We included 118 patients, 35.9% of them were immunosuppressed for different reasons: in 23.7% we isolated SARS-CoV-2 RNA, in 33.1% we identified at least one mycotic agent and both in 15.4%. On BAL we observed in three cases *Aspergillus* spp, in six cases *Pneumocystis* and in 32 *Candida* spp. The prevalence of significant mold infection was 29.3% and 70.7% of cases were false positive or clinically irrelevant infections. In-hospital mortality of patients with fungal infection was 15.3%. The most frequent computed tomography (CT) pattern, evaluated with the Radiological Society of North America consensus statement, among patients with a mycotic pulmonary infection was the atypical one ( $p < 0.0001$ ). Mycotic isolation on BAL may be interpreted as an innocent bystander, but its identification could influence the prognosis of patients, especially in those who need invasive investigations during the COVID-19 pandemic; BAL plays a fundamental role in resolving clinical complex cases, especially in immunosuppressed patients independently from radiological features, without limiting its role in ruling out SARS-CoV-2 infection.

## 1. Introduction

The final diagnosis of severe acute respiratory coronavirus 2 (SARS-CoV-2) infection is based on virus positivity on real-time reverse-transcriptase-polymerase chain reaction (RT-PCR) processed on nasopharyngeal swabs (NP) [1–3]. However, this method is characterized by a high rate of false

negative results, with a sensitivity ranging between 42% and 83% [4]. Due to bronchoalveolar lavage (BAL) low positivity rate for SARS-CoV-2 identification [5–7], its use in the diagnosis of SARS-CoV-2 infection should be reserved only to those cases with indeterminate or atypical radiological computed tomography (CT) manifestations with high clinical suspicion [8].

In a pandemic setting fungal pulmonary infections may be misdiagnosed as SARS-CoV-2 pneumonia. The symptoms, such as fever, cough and dyspnoea are notoriously similar [9, 10], and even the radiologic appearance may be difficult to distinguish, since a lot of atypical coronavirus disease 2019 (COVID-19) radiologic signs can mimic mycotic infections like invasive pulmonary aspergillosis [11].

It is rising evidence that COVID-19 patients have a high incidence of fungal complications, in particular aspergillosis, candidemia and pneumocystosis [12, 13]. COVID-19-associated invasive pulmonary aspergillosis (CAPA) affects nearly 30% of critically ill patients and it is associated with higher mortality rates [14]. Moreover, critically ill COVID-19 patients are exposed to other mycotic pathogens such as *Candida* species and *Pneumocystis jirovecii*: the incidence of these isolations among COVID-19 patients is variable in literature and their etiological value is still debated [15].

Unlike COVID-19, many mycotic infections may benefit from a timely and targeted pharmacological treatment, and patients with a fungal superinfection in COVID-19 pneumonia have poorer outcomes [16, 17]. Therefore, recognizing and treating these life-threatening complications is particularly relevant.

The aim of our study is to describe the incidence of fungal pathogens isolated on BAL among the patients admitted in internal medicine wards who underwent bronchoscopy with BAL for the research of SARS-CoV-2 and other pathogens after a double RT-PCR NP test; the 2nd aim is to describe the clinical characteristics and main outcomes of these patients with fungal pathogens.

## 2. Material and methods

We conducted a single centre, retrospective and observational study of consecutive patients undergoing both a chest CT scan with two consecutive NP swabs in our institution, and a bronchoscopy with BAL with the RT-PCR testing for SARS-CoV-2 RNA and microbiologic culture. Data were collected between 16 March and 30 November 2020. Patients underwent bronchoscopy with different indications: inconclusive diagnosis of SARS-CoV-2 infection after previous examinations, concerns about an alternative etiology of respiratory diseases which would alter the management, suspicion of superinfection (viral, bacterial, fungal), bronchial or lung atelectasis caused by mucous plugs [5]. We excluded from the analysis those cases with incomplete or non-retrievable data or with at least one positive NP. The patients who underwent bronchoscopy more than 7 d after the chest CT scan were also excluded. The study was approved by the local Institutional Review Board (approval protocol number CE 97/20) and was conducted in accordance with Strengthening the

Reporting of Observational studies in Epidemiology statement for observational studies [18]. The population included in this study represents the extension of the one included in a previously published study [5].

### 2.1. Bronchoscopy and BAL technique

During flexible bronchoscopy the tip of the instrument is placed in wedge position through the tributary bronchus of the selected segment of lung parenchyma; three 50 ml aliquots of saline are instilled through the bronchoscope and, after each instillation, the lavage fluid is retrieved using a suction pressure. An optimal sampling is the one that allows one to retrieve more than 30% of the instilled fluid. In our study the BAL fluid recovered was used for microbiological analysis, including the research of SARS-CoV-2 RNA [19].

RT-PCR testing was performed on NP and BAL fluid (Xpert (Cepheid)).

After cytospin and direct examination, all samples underwent culture and incubation for bacteria and fungi evaluation; identification of mycotic agents was achieved by mass spectrometry. *Aspergillus fumigatus* and *Pneumocystis jirovecii* DNA were extracted and PCR performed in duplicate: a single positive well was considered as positive. Galactomannan was dosed on BAL; it was determined by enzyme immunoassay and considered as positive, after two determinations performed on the same sample, when the index was equal or greater than one.

### 2.2. Chest CT execution technique

The Chest CT scans were acquired during a single full inspiratory breath hold, with the patient in supine position. We used a 128-slice CT (Philips Ingenuity Core, Philips Healthcare, Netherlands). Scan parameters were: tube voltage: 120 kV; 210 mAs; collimation width 0.625; spiral pitch factor: 1.08; matrix 512 (mediastinal window) and 768 (lung window). The images were subsequently reconstructed with a slice thickness of 1 mm.

### 2.3. Recorded data

For each patient the following data were recorded: demographics (age, gender), days from the symptoms' onset, in-hospital stay (hospitalization days, admission unit), last negative NP date, bronchoscopy date, white blood cells count (WBC, in  $\times 10^3 \mu\text{l}^{-1}$ ), C-reactive protein (CRP, in  $\text{mg dl}^{-1}$ ) and procalcitonin (PCT, in  $\text{ng ml}^{-1}$ ) blood levels. Information obtained from the bronchoscopy were recorded in each patient: mycotic isolation, SARS-CoV-2 positivity/negativity, other respiratory viruses and bacteria detected. We also noted the laboratory data on peripheral blood (blood cells count, CRP, PCT) and the main CT alterations (bilateral, peripheral, posterior or multilobar involvement, presence of ground-glass opacities, consolidations, crazy paving, reversed halo sign pleural or pericardial effusions or lymph

node enlargements). For each patient we calculated, at Emergency Department admission, the Novara-COVID score for the stratification of in-hospital clinical instability and mortality of patients with suspected COVID-19 [20].

Each CT was evaluated by two expert radiologists (ZF and FF) and for each case the likelihood of COVID-19 pneumonia was reported, based on Society of Thoracic Radiology/Radiological Society of North America (STR/RSNA) standards [4], which provides for four categories (typical, indeterminate, atypical appearance and negative for pneumonia).

For the diagnosis of aspergillosis and other mold infections, we used the European Organization for Research and Treatment of Cancer (EORTC)/Mycosis Study Group (MSG) criteria for patients affected by immunosuppressive underlying conditions [21]. Other non-immunosuppressed patients were classified as having a putative invasive pulmonary mold infection or false positive/clinically non relevant colonization: in particular, those patients with positive antigen test or PCR without a positive culture on BAL fluid and without clinical deterioration (stability or improvement of symptoms, stability or reduction of pulmonary infiltrates and inflammatory markers) or correlated to mold infection were considered as false positive or clinically non relevant colonization [12].

## 2.4. Statistical analysis

Descriptive statistical analyses were conducted. We reported frequency and percentage mean and standard deviation (SD) or median and interquartile range (IQR) for categorical and continuous variables. Association between CT pattern and BAL isolation was evaluated using Chi Square or Fisher Exact test as appropriate.

## 3. Results

Between 16 March and 30 November 2020, a total of 118 subjects underwent BAL with search of SARS-CoV-2 RNA and in 33.1% ( $n = 39/118$ ) of them we isolated at least one mycotic agent while in 23.7% ( $n = 28/118$ ) we identified SARS-CoV-2. The 39 included patients were predominantly male ( $n = 27$ , 69.2%) and had a median age of 71.7 years (IQR 60.0–80.7). Most of them were admitted in an internal medicine ward (69.2%); they were hospitalized and underwent bronchoscopy for a median time of 3 (IQR 2–5) and 7 d (IQR 5–13) after symptoms' onset. Mean Novara-COVID score was 3.66 (SD  $\pm$  0.77). Almost all patients were symptomatic for respiratory tract infection (94.9%) with increased inflammatory markers (mean CRP and PCT respectively 9.58 mg dl<sup>-1</sup> and 2.18 ng ml<sup>-1</sup>). According to STR/RSNA standards for SARS-CoV-2 pulmonary infection, chest CT was defined as: typical 7.7%, indeterminate 28.2%, atypical appearance 56.4% and negative for pneumonia 7.7% (table 1).

**Table 1.** Patients' characteristics.

	N (%)
Demographics of patients with an isolated mycotic species	39
Gender	
Female	12 (30.8)
Male	27 (69.2)
Age, years	
<65	15 (38.5)
65–75	8 (20.5)
>75	16 (41.0)
Median [IQR]	71.67 [60.01–80.65]
Hospital unit	
Internal medicine ward	27 (69.2)
Infectious diseases ward	6 (15.4)
Other non ICUs	6 (15.4)
Symptomatic	
Yes	37 (94.9)
Days from symptoms' onset to bronchoscopy	
Median [IQR]	7 [5–13]
Days from NP to hospitalization	
Median [IQR]	3 [2–5]
Days from NP to bronchoscopy	
Median [IQR]	2 [1–3]
Blood analysis	
White blood cell count (in $\times 10^3 \mu\text{l}^{-1}$ ), mean [ $\pm$ SD]	9.58 [ $\pm$ 5.42]
CRP (in mg dl <sup>-1</sup> ), mean [ $\pm$ SD]	12.10 [ $\pm$ 9.41]
PCT (in ng ml <sup>-1</sup> ), mean [ $\pm$ SD]	2.18 [ $\pm$ 4.25]
NOVARA-COVID score	
Mean [ $\pm$ SD]	3.66 [ $\pm$ 0.77]
STR/RSNA CT pattern	
Negative for pneumonia	3 (7.7)
Atypical appearance	22 (56.4)
Indeterminate appearance	11 (28.2)
Typical appearance	3 (7.7)

**Abbreviations:** BAL: bronchoalveolar lavage, CT: Computed Tomography, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, STR-RSNA: Society of Thoracic Radiology—Radiological Society of North America

Comorbidities are reported in table 2. Fourteen patients (35.9%) were immunosuppressed for different conditions: seven cases of immunosuppression for stem cell transplantation, three patients affected by onco-hematological disorders, three patients in chronic steroid treatment for rheumatological diseases (two vasculitis and one case of systemic sclerosis), one renal transplanted patient.

Among the 118 included patients who underwent bronchoscopy for SARS-CoV-2 search, BAL was positive for SARS-CoV-2 in 28 cases (23.7%); as reported above, we isolated at least one mycotic agent in 39 cases (in two patients we isolated two fungi each, for a total of 41 isolations). Eight cases of mycotic and bacterial coinfections were reported. Other viruses and bacteria present on BAL are reported in table 3.

Prevalence of SARS-CoV-2 infection among patients with at least a fungal isolation was 15.4%

**Table 2.** Patients' comorbidities.

	N (%)
Immunosuppression	14 (35.9)
– Stem cell transplantation	7
– Oncohematological disorders	3
– Rheumatological disease in chronic treatment with steroids	3
– Renal transplantation	1
Hypertension	17 (43.6)
Ischemic heart disease	4 (10.2)
Cardiac arrhythmias	9 (23.1)
Diabetes	5 (12.8)
Dyslipidaemia	8 (20.5)
Other oncologic disorders	10 (25.6)
Chronic kidney failure	6 (15.4)
Neurologic disorders	4 (10.2)
Chronic respiratory diseases	5 (12.8)

**Table 3.** Viruses, bacteria and fungi isolated on BAL.

	N
Bronchoscopies performed	118
Virus	
SARS-CoV-2	28
Rhinovirus	4
Metapneumovirus	1
RSV	3
HSV6	8
HSV1	3
CMV DNA	3
EBV DNA	3
Fungi	
<i>Aspergillus fumigatus</i>	3
<i>Pneumocystis jirovecii</i>	6
<i>Candida</i> spp	32
Bacteria	
<i>E. coli</i>	2
<i>P. aeruginosa</i>	2
<i>Klebsiella pneumoniae</i>	2
<i>S. aureus</i>	1
<i>Serratia marcescens</i>	1

**Abbreviation:** CMV: cytomegalovirus, EBV: Epstein-Barr virus, HSV1: herpes simplex virus 1, HSV6: herpes simplex virus 6, RSV: respiratory syncytial virus, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

( $n = 6/39$ ): in these patients, STR/RSNA radiological patterns were defined as typical, atypical and indeterminate in two cases each. On the other hand, the prevalence of bacterial infection in the same group was 20.5% ( $n = 8/39$ ). Finally, the prevalence of other viral agents (at least one virus per patient) was 17.9% ( $n = 7/39$ ).

Among the 39 patients included in the analysis, we isolated 41 molds: three *Aspergillus fumigatus*, six *Pneumocystis jirovecii* and 32 *Candida* spp (table 3). Most ( $n = 4/6$ , 66.6%) of *Pneumocystis jirovecii* cases occurred in patients with previous immunosuppressive underlying conditions, one case in a patient under pre-emptive steroid treatment for SARS-CoV-2

infection: all these cases were appropriately treated; finally, one case was considered as false positive or clinically non relevant. Two of the three cases of *Aspergillus fumigatus* positivity occurred in immunosuppressed patients and received specific treatment; the remaining case was considered as clinically non relevant. Most fungal isolations ( $n = 32$ ) were represented by *Candida* spp: among these, nine cases ( $n = 9/32$ , 28.1%) were detected in immunosuppressed patients and five cases ( $n = 5/32$ , 15.6%) in patients receiving steroids for pre-emptive treatment of SARS-CoV-2 infection. Only five cases ( $n = 5/32$ , 15.6%) underwent specific treatment of the infection, the other cases were considered as clinically irrelevant. With these results, according to EORTC/MSG criteria, the prevalence of mold infections was 29.3% with 12 cases of mycotic infections and 29 false positive or clinically irrelevant infections ( $n = 29/41$ , 70.7%).

Patients with a mycotic infection had a more frequently atypical STR/RSNA CT radiological pattern (60.6%) than patients with SARS-CoV-2 whose most frequent pattern was typical (63.6%). Among patients with both a mycotic isolation and SARS-CoV-2 infection, the three patterns (typical, indeterminate and atypical) were homogeneously distributed ( $p < 0.0001$ ) (table 4).

Among the six patients who were co-infected by SARS-CoV-2 and a mycotic agent, *Candida* spp was the only co-infectant microbe: three of these cases occurred in immunocompromised patients, while one patient was in treatment with preemptive high doses of steroids for a suspected SARS-CoV-2 infection. The mean WBC count and CRP of these six subjects were  $8.42 (\pm 3.44) \times 10^3 \mu\text{l}^{-1}$  and  $12.63 (\pm 7.21) \text{ mg dl}^{-1}$ , respectively.

At the time of BAL, at least one antibiotic treatment was ongoing in most of the included patients: only seven patients ( $n = 7/39$ , 17.9%) were naïve for any antimicrobial treatment. The most frequently used antibiotics were azithromycin (18 times), ceftriaxone (15 times), piperacillin-tazobactam (eight times), amoxicillin-clavulanate (three times), fluoroquinolones (four times).

In-hospital mortality in patients with a fungal infection detected by BAL was 15.3% ( $n = 6/39$ ) and occurred on average at 22.33 d after hospital admission (SD  $\pm 12.3$ , 95% IC 9.5–35.2, median 23.0); three patients were immunosuppressed with mycotic infections and three with false positive or clinically irrelevant infection. Only one of them presented the SARS-CoV-2 co-infection. Among patients who died only in one case BAL evidenced a bacterial co-infectant (*Klebsiella pneumoniae*). For completeness, the mycotic and viral (non SARS-CoV-2) coinfection of detected seven times (7/39, 17.9%): in three cases patients died during hospitalization and previously isolated viruses were Respiratory Syncytial Virus,

**Table 4.** STR/RSNA CT pattern in mycotic, SARS-CoV-2 and combined (fungi and SARS-CoV-2) BAL isolation: *N* (%).

	Fungi ( <i>N</i> = 33)	SARS-CoV-2 ( <i>N</i> = 22)	Fungi + SARS-CoV-2 ( <i>N</i> = 6)
STR/RSNA CT pattern			
Negative for pneumonia	3 (9.1)	0 (0.00)	0 (0.0)
Atypical appearance	20 (60.6)	3 (13.6)	2 (33.3)
Indeterminate appearance	9 (27.3)	5 (22.8)	2 (33.3)
Typical appearance	1 (3.0)	14 (63.6)	2 (33.3)
Outcomes			
In-hospital mortality	5 (16.5)	1 (4.5)	1 (16.6)
In-hospital stay, in days [ $\pm$ SD]	16.56 [ $\pm$ 14.1]	16.76 [ $\pm$ 13.6]	15.66 [ $\pm$ 7.1]

**Abbreviations:** CT: computed tomography, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, STR-RSNA: Society of Thoracic Radiology—Radiological Society of North America

Herpes Simplex Virus-6 and Epstein Barr Virus. None of them was the causative of death.

#### 4. Discussion

In our cohort of patients with suspected pulmonary SARS-CoV-2 infection with two negative RT-PCR test on NP swab we observed a prevalence of fungal pathogens on BAL of 33.1% (39/118), and a positivity for SARS-CoV-2 of 23.7% (28/118). In particular, co-infection of a mycotic agent and SARS-CoV-2 was present in 15.4% (6/39) of cases. According to EORTC/MSG criteria, the prevalence of mold infection was 29.3%, with 70.7% of false positive or clinically irrelevant infections.

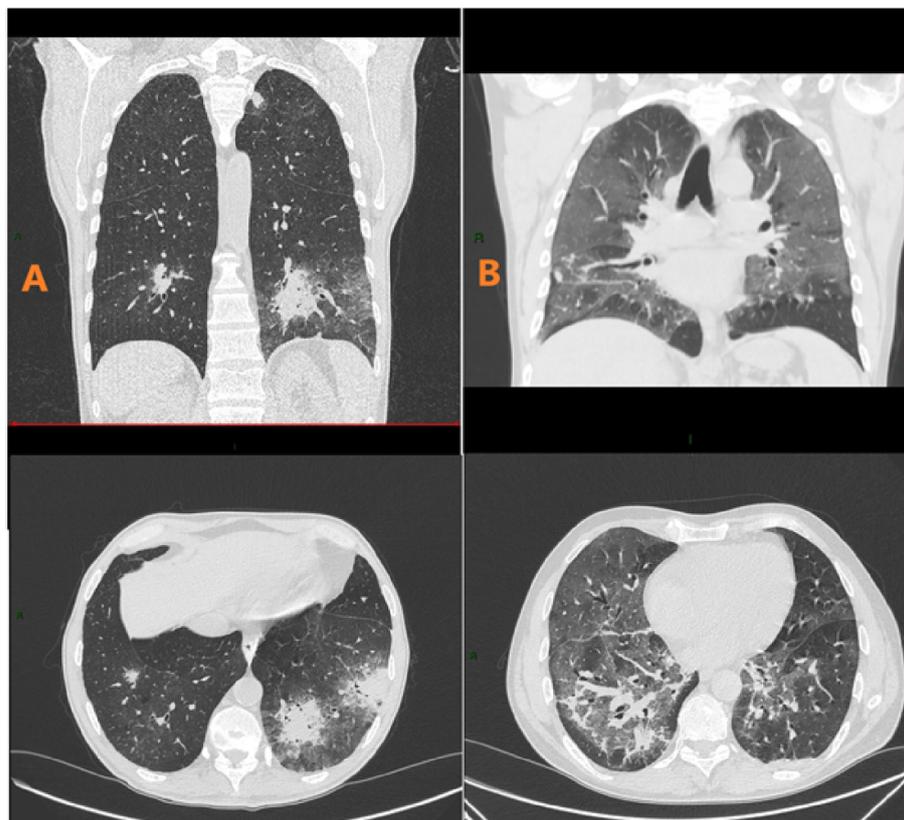
The high mortality rate related to COVID-19 acute respiratory distress syndrome is linked to other infectious complications, such as fungal infections [22]. Recently, it has been demonstrated that patients affected by severe SARS-CoV-2-related pneumonia, without underlying immunosuppression, have a low risk of pulmonary invasive fungal infection, when caused by *Aspergillus spp* [12]. Nevertheless, up to now only few and no specific data are present in the literature regarding the prevalence of mycotic infections among patients who are hospitalized for suspected SARS-CoV-2 pulmonary infection with pulmonary infiltrates, outside the intensive care unit (ICU). Although in many clinical situations a non-invasive approach can be considered sufficient for the diagnostic work-up [23], some studies have investigated the role of bronchoscopy for diagnosis in COVID-19 patients: most of them confirmed the limited place of BAL in SARS-CoV-2 diagnosis, suggesting a role in resolving diagnostic complex cases [7]. In these studies, mycotic infections represent a relevant part of pathogens isolated on BAL: in the paper of Barberi *et al* among 166 negative BAL for COVID-19, a mycotic agent was identified 38 times (22.9%), with *Aspergillus fumigatus* and *Candida spp* being the most isolated ones [24]. Even in the study of Ora *et al* on 28 patients, the authors found five BAL positivities for *Candida spp*, two for *Pneumocystis jirovecii*

and described six cases with galactomannan  $>0.8$  but without information regarding neither the culture nor CRP [7]. In our cohort, the prevalence of mycotic isolation is in line with the results reported in these studies in patients with the same indication and setting.

Mycotic pulmonary infections are associated with a higher mortality rate for patients with nosocomial infection or complicating respiratory failure [25]; moreover, mycotic and bacterial coinfections increase mortality in severely ill COVID-19 patients [26]. In our cohort we reported an in-hospital mortality rate of 15.3% with increased inflammatory markers; multiple cells and molecules are involved in host's response to fungal infection in the lung, resulting in a complex network of inflammatory pathways [27].

Also, the mycotic infections prevalence we observed is in line with the one reported in studies including patients admitted to ICU for severe COVID-19 pneumonia, mainly caused by *Aspergillus* [12, 14, 28, 29]. These similar results, despite being reported in different settings, could support some assumptions:

- SARS-CoV-2 can infect immunocompromised patients, a population particularly susceptible to fungal infections, although it may be difficult to differentiate COVID-19 superinfection from pre-existing fungal colonization in this category [12]: the prevalence of immunocompromised patients is generally homogeneous among hospital wards [30] and these patients have worse hospital outcomes with increased risk of clinical deterioration [31–33].
- Viral pneumonia could be complicated by invasive aspergillosis [34, 35]: Fekkar *et al* reported an incidence of CAPA of 4.8% among patients admitted to ICU for severe COVID-19 pneumonia [12]; in our cohort we did not observe cases of CAPA even though we observed two cases of invasive aspergillosis who required the specific treatment. This fact probably confirms that both hospitalization and underlying clinical



**Figure 1.** Column (A): the chest CT scan of a 60 year old male patient shows bilateral consolidative areas of rounded morphology, encircled by a diffuse ground glass opacity (GGO) halo. The CT was classified as indeterminate according to STR/RSNA probability score. BAL identified *Aspergillus fumigatus* spp and the final diagnosis was angioinvasive aspergillosis. Column (B): the chest CT scan of a 48 year old male shows diffuse bilateral GGO with some consolidative bands in the inferior lobes and no clear peripheral predominance. There is an evident subpleural sparing. The CT was classified as indeterminate according to STR/RSNA probability score; BAL identified *Pneumocystis jirovecii* and no SARS-CoV-2 RNA. The final diagnosis was *Pneumocystis jirovecii* pneumonia.

conditions (in these cases immunosuppression) could independently identify these patients as a high-risk populations for SARS-CoV-2 infection, in which BAL is fundamental to rule out the viral infection. Moreover, all the three patients had a radiological CT pattern either atypical or indeterminate, which may require further investigations to determine the causative agent.

- (c) The isolation of *Candida* spp in the respiratory tract is usually interpreted as contaminant because of its high isolation rate on BAL, and invasive pneumonia is usually a rare complication [36]; nevertheless, even when considered as an innocent bystander, *Candida* may contribute to the worsening of underlying respiratory disease, especially in critically ill patients, prolonging ICU stay and hospitalization [37]. In our cohort, patients with coinfection of SARS-CoV-2 and *Candida* spp had a higher even if non-significant longer hospital stay compared to those who had only COVID-19 without other co-infectants ( $15.0 \pm 7.7$  vs  $17.3 \pm 15.2$  d,  $p = 0.75$ ). However, we must acknowledge that this result could be influenced by the low number of subjects included in the study.

Interestingly, among patients with mycotic isolation on BAL, only six were in pre-emptive treatment with steroids (oral prednisone or intravenous methylprednisolone) for a suspected SARS-CoV-2 infection, which could have promoted the growth of mould on the respiratory tract, independently from their immune status.

Our results on a limited number of patients seem to indicate that the STR/RSNA consensus statement [4] is fairly accurate in discriminating lung mycotic infections from SARS-CoV-2 pneumonia, having correctly assigned about 60% of the patients with the former infection to the atypical category and 63% of the patients affected by the latter to the typical pattern. Nevertheless, we believe that the differential diagnosis between fungal infections and COVID-19 remains a challenge even for the experienced thoracic radiologists (figure 1). For example, distinguishing COVID-19 from *Pneumocystis jirovecii* pneumonia is particularly challenging given the predominantly ‘ground glass’ alterations present in both the conditions, while the different perihilar distribution is sometimes hard to evaluate [38]. As far as it concerns the diagnosis of SARS-CoV-2 pneumonia superinfected by a mycotic agent, we think

that our results confirm the opinions expressed by Koehler *et al* [11] about CAPA: currently, radiology alone cannot effectively identify this subset of patients since many atypical COVID-19 findings overlap with several fungal infections. In this setting keep suggesting that performing a BAL on patients with two consecutive NP RT-PCR tests who have a clinically suspected SARS-CoV-2 infection and a radiological atypical/indeterminate pattern (clinically suspected, according with radiologic images and bio-humoral findings) may help to confirm or rule out this dangerous complication.

Our study has some limitations: first, the retrospective nature of the study, with patients presenting to the Emergency Department where they performed a CT scan and were stratified for the risk of SARS-CoV-2 infection at different stages of the disease. Second, the number of patients with a SARS-CoV-2 and mycotic co-infection is small, limiting the possibility to draw definitive conclusions in these patients hospitalized in a non-intensive care setting. However, to our knowledge this is the first published study that investigates the prevalence of mycotic co-infectants in patients hospitalized in an internal medicine ward, undergoing bronchoscopy with BAL for the research of SARS-CoV-2. All the subjects included were categorized on the basis of their immune status and mycotic infection defined as clinically relevant or irrelevant on the basis of recent guidelines, even if retrospectively [21].

However, our results confirm that the STR/RSNA CT appearance suggests, even if not definitively, the probability of SARS-CoV-2 infection, as well as it determines the need to proceed with further examinations in those cases with indeterminate or atypical appearance [8].

## 5. Conclusions

Mycotic isolation on lower respiratory tract may be interpreted as an innocent bystander, but its identification on BAL could negatively influence the prognosis of patients, especially COVID-19 patients. CAPA and other invasive mycotic infection (especially *Candida* spp), in patients affected by SARS-CoV-2 pneumonia, represent a fearsome complication that should be investigated, particularly in immunocompromised hosts, independently from CT radiological features. We emphasize the role of BAL in resolving clinical complex cases, without limiting its role in ruling out SARS-CoV-2 infection.

## Data availability statement

The data generated and/or analyzed during the current study are not publicly available for legal/ethical reasons but are available from the corresponding author on reasonable request.

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