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Research paper

Invasive pulmonary aspergillosis in COVID-19 critically ill patients: Results of a French monocentric cohort

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ABSTRACT

Introduction. – Coronavirus disease 2019 or COVID-19 is a new infectious disease responsible for potentially severe respiratory impairment associated with initial immunosuppression. Similarly to influenza, several authors have described a higher risk of fungal infection after COVID-19, in particular for invasive pulmonary aspergillosis. The main objective here is to define the prevalence of invasive pulmonary aspergillosis (IPA) in a cohort of COVID-19 patients with moderate to severe acute respiratory disease syndrome (ARDS).

Material and methods. – We conducted a large monocentric retrospective study investigating all the ventilated COVID-19 patients with ARDS hospitalized at Valenciennes' general hospital, France, between March 15, 2020 and April 30, 2020. In the center a systematic IPA screening strategy was carried out for all ARDS patients, with weekly tests of serum galactomannan and beta-D-glucan. Bronchoalveolar lavage with culture and chest CT scan were performed when the serum assays were positives.

Results. – A total of 54 patients were studied. Their median age was 65 years, and 37 of the patients (71%) were male. Two patients had chronic immunosuppression and among all the patients, only 2 non-immunocompromised presented a putative IPA during their stay.

Conclusion. – The prevalence of IPA in this cohort of COVID-19 patients (3.7%) is not higher than what is described in the other ARDS populations in the literature. These results are however different from the previous publications on COVID-19 patients and must therefore be confirmed by larger and multicentric studies.

1. Introduction

SARS-CoV-2 is a new coronavirus responsible for pulmonary infection. It has firstly appeared in December 2019 in the Hubei region in China and has rapidly spread around the world creating a real pandemic. This Coronavirus 2019 disease, also known as COVID-19, mainly causes respiratory damage and is responsible in its most severe forms for acute respiratory distress syndrome (ARDS) [1].

Patients with severe forms of COVID-19 have deep immunosuppression characterized by deep lymphopenia in the initial stage. Similarly to influenza infections, several authors have mentioned the possibility of a more frequent association of invasive fungal infections (IFI), and in particular invasive pulmonary aspergillosis (IPA) in these patients [2].

The main objective of this work is to define in a retrospective analysis, the prevalence of putative IPA in a cohort of COVID-19 patients with moderate to severe ARDS. The modified definition from the *Asp*ICU algorithm developed by Schauwvlieghe et al. was used for this diagnosis of putative IPA in non-immunocompromised patients; it combines clinical, radiological and mycological criteria [3].

2. Material and methods

The cohort took place in the intensive care unit (ICU) at the Valenciennes' general Hospital between the 15thof March 2020 and the 30th of April 2020. All the COVID-19 patients under mechanical ventilation with moderate or severe ARDS were studied. The diagnosis of COVID-19 was systematically performed

Abbreviations: ARDS, Acute respiratory distress syndrome; BAL, Bronchoalveolar lavage; BDG, Beta-D-glucan; BMI, Body mass index; EORTC, European Organization for Research and Treatment of Cancer; GM, Galactomannan; ICU, Intensive care unit; IFI, Invasive fungal infection; IPA, Invasive pulmonary aspergillosis; SD, Standard deviation; TA, Tracheal aspiration.

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by Real-time PCR on a nasal swab or a tracheal aspirate (TA). And an initial chest computed tomography (CT) scan was performed routinely to diagnose the severity of the lesions. Moderate or severe ARDS were both identified using the Berlin definition [4]. The patients' demographic data and comorbidities, principally criteria of immunosuppression and host risk factors, were collected according to the standards of the European Organization for Research and Treatment of Cancer (EORTC) [5].

All the critically ill Covid-19 patients were granted the same protocol of care given to the critically ill patients with influenza. In addition to the first recommendations of Gangneux et al. on COVID-19 patients, beta-D-glucan (BDG) (Fungitell® Associates of Cape Cod, Inc.) and galactomannan (GM) (Platelia® Aspergillus Bio-rad, Marnes-la-Coquette, France) serum assays were performed several times; 48 hours after initiation of invasive mechanical ventilation and then weekly over the duration of the ICU stay [2]. All mechanically ventilated patients also had weekly tracheal aspiration (TA) with bacterial and mycological examination.

In case of positive value of GM (positive at ≥ 0.5 in serum) whenever it was possible, bronchoalveolar lavage (BAL) was performed with direct detection of hyphae, GM assay (positive at ≥ 0.8 in BAL) and culture for *Aspergillus*. A chest computed tomography (CT) scan was also conducted.

Putative IPA in non-immunocompromised patients was only considered after combining the clinical, radiological and mycological criteria defined by Schauwvlieghe et al. [3]. For the mycological criteria, one or more had to be present between a GM index positive on serum, a GM index positive on BAL, a direct microscopic evidence of hyphae and/or a positive *Aspergillus* culture from tissue or a BAL. Based on this practice, we do not perform systematic PCR on serum or BAL to detect *Aspergillus* in the center.

The data are reported as percentages for categorical variables and as a mean ± standard deviation (SD) or median with a range (min-max), as appropriate, for continuous variables.

This observational study was carried out in accordance with the ethical principles reflected in the Declaration of Helsinki and was approved by the "Comité de Protection des Personnes Nord-Ouest IV" which is the Institutional Review Board of the University Hospital of Lille (IORG0009553) under the number HP 20/29[6].

3. Results

A total of 54 COVID-19 patients with moderate to severe ARDS under mechanical invasive ventilation were included. 37 of them were male (71%) with a median age of 65 years. Two patients were immunocompromised; one underwent a liver transplant and the other was followed for myeloma, but neither has developed an IFI during their stay. 15 patients (28%) have died. Demographic data and clinical characteristics are presented in Table 1.

Two patients showed early putative IPA, neither had prior immunosuppression or host risk factors (Table 2).

The first patient was 55 years old. He was immediately hospitalized in our intensive care unit with deep hypoxemia, seven days after the beginning of his symptoms. The first CT scan carried out in an emergency revealed serious COVID lesions, with approximately 75% of the lung parenchyma affected. There were no pulmonary aspergillosis lesions on the examination. Corticosteroid therapy at a rate of 2 mg/kg/day was started for the treatment of COVID-19 since day 1. With a neutrophil count of 6400/mm^3 at the first biology, no neutropenia was found during the stay.

The serum GM on day 2 was immediately positive with a value of 3.93. The BAL performed on day 5 did not show any hyphae on direct examination but *Aspergillus fumigatus* was positive on culture and GM rate was 2.46 on the BAL. *Pseudomonas aeruginosa* was also positive on the BAL culture but other researches on IFI and viral infections ended up being negative. The control chest CT scan was only performed on day 15 and was highly suggestive of an IPA with the appearance of numerous excavations at the level of the pulmonary apex associated with typical disseminated nodular lesions. Treatment with voriconazole was started as soon as the first serum dosages were received, on day 3. The clinical evolution of the patient was however unfavorable. The serum GM concentration continued to rise despite treatment, from 5.8 on day 7 to >6 on day 14; BDG concentration also kept on increasing, it went from 62 pg/ml on day 2, to 203 pg/ml on day 7 and 1783 pg/ml on day 14. Brain death occurred on day 18 after a massive cerebral hemorrhage, probably due to mycotic aneurysm rupture.

Table 1
COVID-19 associated ARDS patients' demographic characteristics and underlying diseases at ICU admission.

Items	$n=54$
Age, years (range)	65 (44-83)
Sex, Male *n* (%)	39 (72.2%)
BMI (kg/m^2) mean ± SD	32.7 ± 6.2
Charlson comorbidity index mean ± SD	3.1 ± 1.9
Comorbidities *n* (%)	
Diabetes mellitus	22 (40.7%)
Chronic immunosuppression	2 (3.7%)
Chronic kidney disease	4(7.4%)
Chronic respiratory failure	2(3.7%)
Chronic liver disease	2 (3.7%)
Cardio vascular disease	10 (18.5%)
Covid-19 lesions on initial chest CT-scan *n* (%)	
[<25%]	6 (11.1%)
[25–50%]	16 (29.6%)
[50-75%]	21 (38.9%)
[>75%]	11 (20.4%)
Vasopressors *n* (%)	52 (96.3%)
Prone position *n* (%)	34 (62.9%)
ECMO rescue *n* (%)	4 (7.4%)
Renal replacement therapy *n* (%)	22 (40.7%)
Interval from ICU admission to ICU discharge, days (range)	15,2 (2-42)
Mortality in ICU *n* (%)	15 (27.8%)

BMI: body mass index; SD: standard deviation; ECMO: extracorporeal membrane oxygenation; ICU: intensive care unit.

The second patient was 72 years old. He was hospitalized with pleuropneumonia for ten days before joining the intensive care. No bacteria were found, but the diagnosis of COVID-19 was positive by PCR three days before the transfer to intensive care. The chest CT scan performed on the same day as the SARS-CoV-2 PCR revealed significant COVID lesions, with approximately 50% of the lung parenchyma affected. No pulmonary embolism and no pulmonary aspergillosis lesions were visible on the examination. Corticosteroid therapy was started in intensive care, at a rate of 2 mg/kg/day. The neutrophil count was of 5650/mm^3 at the first biology, no neutropenia was found during the hospitalization.

His serum GM rate was negative on day 2 in intensive care (0.07) but positive on day 7 (0.78) with a BDG at 16 pg/ml on day 2 and 706 pg/ml on day 7 (positive at ≥ 80 pg/ml). *Aspergillus fumigatus* was identified in TA culture performed on day 8, but the BAL was not possibly done because of the severity of hypoxemia. The chest CT scan performed on day 10 suggested severe IPA with the appearance of multiple excavated lesions of the apex and centrolobular nodular lesions with a halo sign. Voriconazole treatment was started. No bacterial or viral infections (other than SARS-CoV-2) were documented for this patient during his stay. The kinetic of serum GM and BDG decreased under treatment, respectively to 0.11 and 513 pg/ml on day 15. But the patient yet died due to a shock and mulivisceral failure on day 18. The chronology of highlights of these two patients is presented in Table 3.

Table 2
Characteristics of patients with putative invasive pulmonary aspergillosis.

	Patient 1	Patient 2
Risk factors of severe COVID-19	Moderate overwieght.	Moderate overweight, hypertension, diabetes.
Pre-existing pulmonary pathology	No	No
Risk factors of IPA[a]	No	No
Specific anti COVID-19 therapy	Hydroxychloroquine	Tocilizumab
Steroids use, 2 mg/kg/day	Yes	Yes
Vasopressor	Yes	Yes
Prone position	Yes	Yes
ECMO rescue	No	No
Renal replacement therapy	Yes	Yes
IPA diagnosis [3]	Fever refractory, deep hypoxemia	
Clinical criteria	3.93	Recrudescent fever, worsening respiratory insufficiency
Serum galactomannan index	2.46	0.78
BAL galactomannan index	*Aspergillus fumigatus*	-
BAL or TA culture	62	*Aspergillus fumigatus*
B-D-glucan, pg/ml	Excavations, nodular lesions	706
Chest CT scan		Excavations, nodular lesions with halo sign
Outcome	Death	Death

IPA: invasive pulmonary aspergillosis; ECMO: extracorporeal membrane of oxygenation; BAL: bronchoalveolar lavage; TA: tracheal aspirate; CT: computed tomography.
[a] The risk factors of IPA have been defined by European Organization for Research and Treatment of Cancer (EORTC) and include: deep neutropenia for more than 10 days, Hematologic malignancy, receipt of an allogenic stem cell transplant or a solid organ transplant, daily use of corticosteroids for more than 3 weeks, severe acute graft-versus-host disease and treatments with recognized T-cell or B-cell immunosuppressants [5].

Table 3
Chronology of highlights of the two patients with putative IPA.

	Patient 1	Patient 2
COVID-19 diagnosis by PCR	Day 0	Day 0
Hospitalisation in intensive care	Day 0	Day 3
Mechanical ventilation	Day 0	Day 3
Start of corticosteroid treatment	Day 1	Day 3
Galactomannan positivity on serum assay	Day 2	Day 10
Tracheal aspirate or bronchoalveolar lavage	Day 5	Day 11
Chest computed tomography scan	Day 15	Day 13
Death	Day 18	Day 21

Three patients had elevated serum BDG without increasing GM, they were not immunocompromised. Two of them had systemic candidiasis due to *Candida albicans* and one had *Candida tropicalis* fungemia. No *Aspergillus* were detected in these three patients, nor in all TA realized on the other 49 patients during their stays.

4. Discussion

We have reported 2 cases of putative IPA in the 52 immunocompetent COVID-19 patients with moderate to severe ARDS (and 2 immunocompromised patients without IPA). These two patients with putative IPA received corticosteroid therapy for the treatment of their COVID-19 but this treatment was started less than 3 weeks (2 days and 7 days) before the diagnosis of IPA and therefore could not be considered a risk factor for the host for API [5].

The frequency of putative IPA in this large monocentric cohort is therefore relatively low (3.7%) and seems similar to what is described with severe ARDS in post-infectious pneumopathy. Schauwvlieghe et al. in fact, have noticed a rate of 5% in their retrospective series involving 315 immunocompetent ICU patients with severe ARDS [3]. And even if our cohort lacks potency, the rate of putative IPA in COVID-19 does not appear to be as elevated as in populations with influenza ARDS. Indeed, the rate of secondary IPA was 14% in the retrospective analysis of Schauwvlieghe et al. established between 2009 and 2016, where 45 cases of secondary IPAs were described among 315 immunocompetent patients with influenza ARDS [3].

The publication by Chen et al. which presents a cohort of 99 COVID-19 patients during January 2020 at the Jinyintan Hospital in Wuhan also shows a low frequency of IPA with only one positive patient for *Aspergillus flavus*, without specifying whether this was a colonization or a true IPA [7]. We should highlight that in Chen et al.'s study only 17 patients were described with ARDS; the other patients had a less severe form of COVID-19.

The feedback recently published by van Arkel et al. differs from ours [8]. The authors report a cohort of 31 COVID-19 patients on mechanical ventilation during the months of February and March 2020; of which 6 patients (19.4%) developed a presumed secondary IPA, 5 of these had evidence of *Aspergillus fumigatus* in tracheal aspirations. However, only 3 of the 6 patients had a positive GM level indicating probable IPA when the other 3 patients had neither no GM level nor negative serum levels. This does not enable to distinguish a possible IPA from a simple *Aspergillus* colonization. Furthermore, only one patient had a chest CT-scan and no IPA-suggestive images were found on the exam. It is interesting to note that for the 3 patients with probable IPA, the delay of IPA diagnosis after ICU admission for severe COVID-19 was 5 days. This rapid onset is similar to the one observed for the two patients in Valenciennes' hospital for who the secondary IPA was done very early in the event of severe SARS-CoV-2 infection.

Another recent publication by Alanio et al. reports 8 putative IPAs and one probable IPA in 27 mechanically ventilated patients with COVID-19 [9]. In this publication, putative IPA was considered if *Aspergillus spp* were identified in BAL culture, or if there were two conditions met between the presence of *Aspergillus spp* in TA; a positive *Aspergillus fumigatus* quantitative real-time PCR in BAL, TA or serum; a positive galactomannan detection in BAL or serum; and a positive BDG in serum. For these 8 putative IPAs, only one had a positive GM in BAL (negative in serum) with *Aspergillus fumigatus* identification in the BAL culture. Two patients had positive identification of *Aspergillus* in BAL culture with negative GM in BAL and serum; and two patients had negative BAL culture, but one had positive GM in BAL and the other, positive GM in serum. We cannot deny that they could all be putative IPAs but most only had one mycological criteria for it. In opposition, in our cohort, we may have missed IPAs by not routinely performing BAL for all patients and using only the GM serum assay in first step to routine performance, which has lower sensitivity than the GM assay on TA or BAL (sensitivity 25 to 65% versus 88% in different series of

non-neutropenic patients) [3,10]. But although we did not perform systematic BAL, all patients had TA cultures during their stay and none were positive for *Aspergillus spp* when the serum GM assays were negative.

To our knowledge while our cohort is a single-center study, it is one of the largest publications of critically ill COVID-19 patients with systematic search for IPA by repeated serum assays. And even if it lacks potency, the prevalence of putative IPA in patients with COVID-19 does not seem to be greater than in other situations of severe pulmonary sepsis and is not as elevated as in critically ill patients with influenza. These results need to be confirmed by larger studies of patients with severe COVID-19, and the mechanisms of action explaining such difference with influenza could only be advanced after having acquired a better knowledge of the physiopathology of SARS-CoV-2.

5. Conclusion

The new Coronavirus 2019 disease might be responsible for severe forms of ARDS but the risk of secondary IPA does not appear to be more significant than in other infectious ARDS in immunocompetent patients. For this retrospective study doesn't demonstrate an increased prevalence of putative IPA. Given the high morbidity of IPA in critical care patients, these results need to be confirmed by large cohorts.

Ethics approval and consent to participate

This observational study was carried out in accordance with the ethical principles reflected in the Declaration of Helsinki and was approved by the "Comité de Protection des Personnes Nord-Ouest IV" which is the Institutional Review Board of the University Hospital of Lille (IORG0009553) under the number HP 20/29.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to the French patient protection law, but are available from the corresponding author on reasonable requests.

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Authors' contributions

M.V., W.Z. and P.S.L. drafted the manuscript. All authors read, revised and approved the final manuscript.

Consent for publication

Not applicable.

Disclosure of interest

The authors declare that they have no competing interest.

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