Diagnosing COVID-19-associated pulmonary aspergillosis

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There is increasing concern that patients with coronavirus disease 2019 (COVID-19) might be at risk of developing invasive pulmonary aspergillosis co-infection.[1] In a cohort of 221 patients with COVID-19 in China, fungal infections were diagnosed in seven individuals, all of whom were admitted to the intensive care unit (ICU).[2] However, causative fungal pathogens were not identified.[2] Given that in China, galactomannan testing is rarely available,[3] the real burden of invasive pulmonary aspergillosis in patients with COVID-19 requiring ICU admission is probably underestimated. Indeed, nine patients with COVID-19 and invasive pulmonary aspergillosis were recently described in France (33% of 27 admitted to the ICU with COVID-19),[4] and five in Germany (26% of 19 admitted);[5] rates similar to those observed in association with influenza.[6] Although serum galactomannan is a sensitive diagnostic marker in patients with neutropenia in intensive care, galactomannan sensitivity was only 25% in patients who did not have neutropenia, but had proven invasive pulmonary aspergillosis.[7] Although serum galactomannan was positive in 65% of patients with influenza-associated pulmonary aspergillosis,[6] only three (21%) of 14 patients with COVID-19-associated pulmonary aspergillosis were serum galactomannan positive.[4,5] Reasons for the lower sensitivity in patients with COVID-19 versus those with influenza are unknown, although treatment with chloroquine might have a negative effect on serum galactomannan performance, because the drug exhibits in-vitro activity against *Aspergillus fumigatus*.[8] Exposure to antifungals is a well known factor that decreases the sensitivity of serum galactomannan testing.

Negative serum galactomannan might indicate that *Aspergillus* spp hyphae are unable to cause angioinvasive growth and release galactomannan into the blood. Most patients with COVID-19-associated pulmonary aspergillosis did not have European Organization for the Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) host factors, because only two (15%) of 13 had haematological malignancy as underlying disease.[3] Absence of host factors was also apparent in influenza-associated disease, in which 57% of patients could not be classified according to the EORTC/MSGERC consensus definition or the *AspICU* algorithm for patients in ICUs. A retrospective multicentre cohort study showed that influenza infection was an independent risk factor for invasive pulmonary aspergillosis.[6] In addition to local erosion of the epithelial barrier of the respiratory tract, influenza virus can exhibit a direct immunomodulatory effect through suppression of the NADPH oxidase complex.[9] Suppression of the NADPH oxidase complex might cause a temporary disease status resembling chronic granulomatous disease, which itself is associated with invasive pulmonary aspergillosis development.[9] Severe COVID-19 is associated with immune dysregulation, affecting both T-helper cell 2 (Th2) and Th1 responses,[10] although this has not been extensively studied and a direct immunomodulatory effect on the known antifungal host defence has not been demonstrated. During the first severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak in 2003, only four cases of proven invasive pulmonary aspergillosis were reported among 8422 probable SARS cases.[3] Because all four invasive pulmonary aspergillosis cases were associated with concomitant corticosteroid therapy, coronavirus infection itself might not increase the risk for invasive pulmonary aspergillosis, but other risk factors might have.

Bronchoalveolar lavage galactomannan testing is important to diagnose invasive pulmonary aspergillosis in the ICU and high galactomannan levels (galactomannan index >2·5) were observed in patients with presumed COVID-19-associated pulmonary aspergillosis.[5] However, only a restricted role for bronchoscopy has been recommended in COVID-19, because it is an aerosol-generating procedure that poses risks to patients and personnel.[11] Collection of upper respiratory samples is the preferred method for diagnosis, and tracheal aspirates and non-bronchoscopic alveolar lavage in intubated patients.[11] Although *Aspergillus* spp can be detected in sputum and tracheal aspirates in patients with COVID-19-associated pulmonary aspergillosis, its presence might reflect oral pharyngeal colonisation because *Aspergillus* spp is considered a core component of the basal oral mycobiome. Furthermore, galactomannan testing is not validated for upper respiratory tract specimens. Bronchoscopy is recommended in COVID-19 only when the intervention is considered lifesaving, which

Panel: **Crucial next research questions for COVID-19 associated invasive pulmonary aspergillosis**

Diagnosis of CAPA

- What is the positive predictive value of culture isolation of *Aspergillus* species in samples from the upper respiratory tract (infections *vs* colonisation)?
- In light of low sensitivity of serum galactomannan, are there alternative blood tests for CAPA? What is the performance of *Aspergillus* PCR, β-D-glucan, and the *Aspergillus*—specific lateral flow device and lateral flow assay?
- Do radiological signs differ in people with CAPA from whats seen in COVID-19 without CAPA

Prophylaxis and treatment of CAPA

- Is the incidence of CAPA in intensive care unit patients high enough to justify antifungal prophylaxis trials?
- What is the clinical relevance of CAPA? Is there a survival benefit with antifungal treatment?
- What is the CAPA-associated mortality? Autopsy study *vs* post-mortem lung biopsy?
- What is the optimal treatment of CAPA? Considerations of efficacy, dosing, adverse events, and drug–drug interactions

Immunology or host factors

- Underlying host factors (neutropenia, lymphopenia, monocytopenia, polymorphims; eg, PTX3, dectin-1, and NADPH-oxidase) or a role for concomitant medication, such as (hydroxy)chloroquine being either harmful (causing defective autophagy) or having direct antifungal or protective effect like in chronic granulomatous disease?
- Role of the kallikrein–kinin system in antifungal host defense
- Is there an underlying antifungal defect caused by COVID-19 or associated with CAPA, such as defective reactive oxygen species production, defective T-helper cell 1 responses, defective LC3-associated phagocytosis, or defective neutrophil extracellular traps activation or release, such as in chronic granulomatous disease?

CAPA=COVID-19-associated pulmonary aspergillosis. COVID-19=coronavirus disease 2019.

includes secondary infectious causes.[11] Radiological and clinical signs of invasive pulmonary aspergillosis in non-neutropenic patients are mostly unspecific and bronchoscopy is thus indicated in critically ill patients with COVID-19 who are suspected of secondary infection, including fungal diagnostic work-up. Even if evidence for *Aspergillus* spp is recovered, uncertainty remains about whether patients truly develop invasive disease and require antifungal therapy. Indeed, eight of the nine patients with COVID-19-associated pulmonary aspergillosis from France were not treated with antifungal drugs, and the three deaths were considered not to be related to aspergillosis, but clinically attributed to bacterial septic shock.[3] Autopsies were not performed to confirm the clinical diagnosis.

It is therefore crucial to gain insight into the interaction between *Aspergillus* spp and the SARS-CoV-2-infected lung (panel). Only histopathology can prove invasive pulmonary aspergillosis through autopsy of deceased patients with COVID-19-associated pulmonary aspergillosis. If autopsy is precluded because of the risk of aerosol formation, post-mortem lung biopsy might be considered as an alternative to obtaining tissue. Until histopathological evidence of COVID-19-associated pulmonary aspergillosis is obtained, we believe that patients with COVID-19 who are critically ill with evidence for *Aspergillus* spp in bronchoalveolar lavage or serum should receive antifungal therapy according to national and international guidelines.

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