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COVID-19-associated *Aspergillus* tracheobronchitis: the interplay between viral tropism, host defence, and fungal invasion

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Invasive pulmonary aspergillosis is emerging as a secondary infection in patients with COVID-19, which can present as alveolar disease, airway disease (ie, invasive *Aspergillus* tracheobronchitis), or both. Histopathology of invasive *Aspergillus* tracheobronchitis in patients with severe COVID-19 confirms tracheal ulcers with tissue invasion of *Aspergillus* hyphae but without angioinvasion, which differs from patients with severe influenza, where early angioinvasion is observed. We argue that aggregation of predisposing factors (eg, factors that are defined by the European Organisation for Research and Treatment of Cancer and Mycoses Study Group Education and Research Consortium or genetic polymorphisms), viral factors (eg, tropism and lytic effects), immune defence factors, and effects of concomitant therapies will determine whether and when the angioinvasion threshold is reached. Management of invasive *Aspergillus* tracheobronchitis should include reducing viral lytic effects, rebalancing immune dysregulation, and systemic and local antifungal therapy. Future study designs should involve approaches that aim to develop improved diagnostics for tissue invasion and airways involvement and identify the immune status of the patient to guide personalised immunotherapy.

Introduction

Invasive aspergillosis is well recognised as a complication of treatment for patients with acute leukaemia or who have had solid organ transplantation or stem cell transplantation.¹ The risk for invasive aspergillosis is strongly associated with neutropenia as a prominent host factor, but the infection is increasingly observed in critically ill patients. Among the increase of patients with invasive aspergillosis in the intensive care unit (ICU), patients with the infection secondary to severe viral illness, notably influenza pneumonia, represent an important group.² Studies noted that influenza was an independent risk factor for invasive aspergillosis, and influenza-associated pulmonary aspergillosis (IAPA) was observed both in patients with susceptibility host factors (panel), as defined by the European Organisation for Research and Treatment of Cancer (EORTC) and Mycoses Study Group Education and Research Consortium (MSGERC),³ and in previously healthy individuals.^{2,4,5} 90-day mortality after ICU admission for patients with IAPA was 51% (42 of 83) compared with 28% (99 of 349) for patients with influenza without IAPA.² Cohort studies show that critically ill patients with COVID-19 are also at high risk of developing invasive aspergillosis.^{6–11} COVID-19-associated pulmonary aspergillosis (CAPA) was observed in 3–33% of (mostly mechanically ventilated) patients who were admitted to ICUs, although various case definitions for CAPA were used.¹² Reported mortality rates for patients with CAPA (ie, 44–74%) are substantially higher than those observed in critically ill patients with COVID-19 without CAPA (ie, 19–39%) and are similar to mortality rates that are reported for patients with IAPA.^{8,9}

One important clinical manifestation of invasive aspergillosis in patients with severe viral pneumonia is invasive *Aspergillus* tracheobronchitis (IATB), which is

Key messages

- Invasive *Aspergillus* tracheobronchitis represents airways disease occurring in up to 56% of patients with severe influenza and in up to 20% of patients with severe COVID-19.
- The ability of *Aspergillus* to cause angioinvasion is hypothesised as a crucial step in the pathophysiology of invasive *Aspergillus* tracheobronchitis (ie, angioinvasion threshold model), which determines diagnostic test performance and disease progression.
- Invasive *Aspergillus* tracheobronchitis presents a highly lethal *Aspergillus* disease manifestation in patients with severe influenza, which occurs early after intensive care unit admission, whereas most invasive *Aspergillus* tracheobronchitis cases develop later after intensive care unit admission in patients with COVID-19-associated pulmonary aspergillosis and often present a less progressive course of disease.
- The aggregation of predisposing factors (eg, presence of European Organisation for Research and Treatment of Cancer and Mycoses Study Group Education and Research Consortium factors or genetic polymorphisms), viral factors (eg, tropism and lytic effects), immune dysregulation, and effects of antiviral or immunosuppressive therapies will determine whether the angioinvasion threshold is reached and when.
- Future study design should involve approaches that are aimed to develop improved diagnostics for tissue invasion and airways involvement and identify the immune status of the patient to guide personalised immunotherapy.

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Panel: European Organisation for Research and Treatment of Cancer and Mycoses Study Group Education and Research Consortium factors

- History of neutropenia (ie, $<0.5 \times 10^3$ neutrophils per L [<500 neutrophils per mm^3] for >10 days) that is temporally related to the onset of invasive fungal disease
- Haematological malignancy
- Receipt of an allogeneic stem-cell transplant
- Receipt of a solid organ transplant
- Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a therapeutic dose of more than 0.3 mg/kg for at least 3 weeks in the past 60 days
- Treatment with other recognised T-cell immunosuppressants, such as calcineurin inhibitors, TNF blockers, lymphocyte-specific monoclonal antibodies, or immunosuppressive nucleoside analogues, during the past 90 days
- Treatment with recognised B-cell immunosuppressants, such as BTK inhibitors (eg, ibrutinib)
- Inherited severe immunodeficiency (eg, chronic granulomatous disease, STAT3 deficiency, or severe combined immunodeficiency)
- Acute graft-versus-host disease grade 3 or 4, involving the gut, lungs, or liver, that is refractory to first-line treatment with steroids

See Online for appendix

characterised by plaques in the large airways (ie, trachea and bronchi). Although a known manifestation of invasive aspergillosis in recipients of lung transplants,¹³ up to 56% of patients with IAPA have been reported to present with IATB.^{4,14} Epithelial erosion due to virus replication is most likely an important predisposing factor to developing IATB, as this factor can provide a portal of entry for *Aspergillus* to cause invasive airway disease. IATB has been reported in 10–20% of critically ill patients with COVID-19 and CAPA.^{8,10} The frequency of IATB in patients with COVID-19 and CAPA might be lower than is observed in patients with IAPA, but it might also be under-reported due to the reluctance of clinicians to do bronchoscopy in patients with COVID-19 during the first wave of the pandemic (ie, approximately December, 2019, to June, 2020). Diagnosing IATB is highly relevant as mortality that is associated with this manifestation was reported to be 90% (9 of 10) in patients with IAPA compared with 44% (11 of 25) in patients with other pulmonary manifestations,¹⁴ whereas mortality data are scarce for patients with severe COVID-19.

Histopathology of illustrative cases

To understand the pathophysiology of IATB in patients with COVID-19, the histology of some patients with proven COVID-19 and IATB was compared with that of patients with influenza and IATB. Figure 1A shows a

tissue fragment from a bronchial biopsy that was obtained from an endobronchial plaque that was observed at bronchoscopy. The patient had no EORTC and MSGERC risk factors and bronchoscopy was done 8 days after ICU admission. Before bronchoscopy, the patient had received dexamethasone at a dose of 6 mg/day for 8 days. The epithelial lining showed partial ulceration, which is most likely due to SARS-CoV-2 replication. ACE2 receptor expression, which serves as an entry point for SARS-CoV-2, is seen in the pre-existent, reactive respiratory epithelium (appendix p 1) and the metaplastic epithelium near the ulcer (appendix p 2). At the ulcer, necroinflammatory debris and several septate hyphae can be identified that are consistent with *Aspergillus* infection (figure 1A). The hyphae show superficial tissue invasion but no deep invasion in the underlying tissue. Furthermore, there was no evidence for angioinvasion. In the stroma, nuclear debris, neutrophils, some macrosialin-positive histiocytes (appendix p 3), and a few CD8-positive cytotoxic T lymphocytes are seen (appendix p 4). A tracheal biopsy from a second patient with COVID-19 and proven IATB shows similar histopathological features (figure 1B). Similar to the first patient, this patient had no EORTC and MSGERC risk factors, and the CAPA diagnosis was made 17 days after ICU admission. Before the biopsy, the patient had received dexamethasone for 10 days at a dose of 6 mg/day and methylprednisolone for 1 day at a dose of 60 mg. Compared with patient 1, more advanced ulceration was found reaching into the perichondral tissue (figure 1B). Staining for the SARS-CoV-2 spike protein did not show any viral protein (appendix p 5). Invasive growth of *Aspergillus* hyphae is seen in figure 1B, but again, there was no evidence for angioinvasion. Furthermore, numerous macrosialin-positive histiocytes and few CD8-positive cytotoxic T cells were found lining the edge of the ulcerative lesion (appendix p 5).

For comparison, Figure 2 shows the histopathology of IATB in a patient with severe influenza. This patient had no EORTC and MSGERC risk factors and was diagnosed with IAPA 3 days after ICU admission. The infection was rapidly fatal and the patient died on day 5 after ICU admission. He had received corticosteroids for vasopressor-resistant shock in the 4 days before death. Extensive ulceration and necrosis in the trachea were noted at autopsy (figure 2A). Multiple *Aspergillus* hyphae can be identified infiltrating the tracheal submucosa. Few inflammatory cells are seen at the ulcer level and in the superficial layers of the trachea. In the adventitia, moderate inflammation with neutrophils admixed with macrophages is seen with necrotic debris. There is clear evidence for invasive growth of *Aspergillus* hyphae in surrounding blood vessels (figure 2B). In addition to invasive growth of hyphae, *Aspergillus fumigatus* vesicles were found in the lumen of the trachea (appendix p 6). These structures are formed by the fungus to allow

sporulation, and *Aspergillus* conidia are visible on the epithelial cells of the trachea.

Pathophysiology of IATB

Invasive aspergillosis is an opportunistic infection that requires a defect of the host defence to develop. Predisposing factors that increase the risk for invasive aspergillosis have been well described in patients with haematological malignancies and include intrinsic factors, such as acute myeloid leukaemia, and extrinsic factors, such as intensive chemotherapy. Both of these factors lead to severe mucositis and reduction in neutrophil counts (ie, granulocytopenia) and thus present a high risk of developing invasive aspergillosis.¹ Additionally, disruption to neutrophil function (eg, caused by corticosteroid treatment or chronic granulomatous disease) predispose people to the development of invasive aspergillosis. The presence of host factors has become an important criterion for classification of patients with invasive aspergillosis and is part of case definitions, such as the EORTC and MSGERC's definition.³ Invasive aspergillosis that is secondary to severe viral infection can occur in patients with EORTC and MSGERC host factors, but 30–78% of patients with IAPA do not have these host factors.^{2,4,5,14} This proportion is even higher in patients with CAPA (86% [123 of 143] of patients do not have these host factors).¹² Although EORTC and MSGERC host factors, and other factors that are not covered by the EORTC and MSGERC case definitions, can contribute to patient susceptibility to CAPA,¹⁵ virus-induced anatomical or immunological changes are likely to have an important role in providing the opportunity to develop invasive aspergillosis.²

Role of the virus

Human adapted influenza A viruses preferentially bind to α 2,6-linked sialic acids.¹⁶ By use of lectins that were specific for sialic acid α 2,6-galactose sialyloligosaccharides, a study showed that epithelial cells in the trachea and bronchi, including terminal and respiratory bronchioles, mainly express sialic acid α 2,6-galactose sialyloligosaccharides (rather than sialic acid α 2,3-galactose sialyloligosaccharides).¹⁷ Histopathological findings from patients with influenza A at autopsy showed multifocal desquamation of the epithelium, congestion, haemorrhage, and necrotising tracheobronchitis.¹⁸ Histopathological changes involving the trachea and bronchi were observed in two-thirds of fatal 2009 H1N1 influenza cases, indicating that airway epithelium is commonly affected during influenza pneumonia.¹⁹

Autopsy studies of patients who died from COVID-19 showed plaques in the trachea and large bronchi and microscopy showed mucosal ulceration with mixed inflammatory cell infiltration, including neutrophils and fibrin.²⁰ In one patient, SARS-CoV-2 immunohistochemical staining and electron microscopy confirmed the presence of SARS-CoV-2-like particles in tracheal epithelial

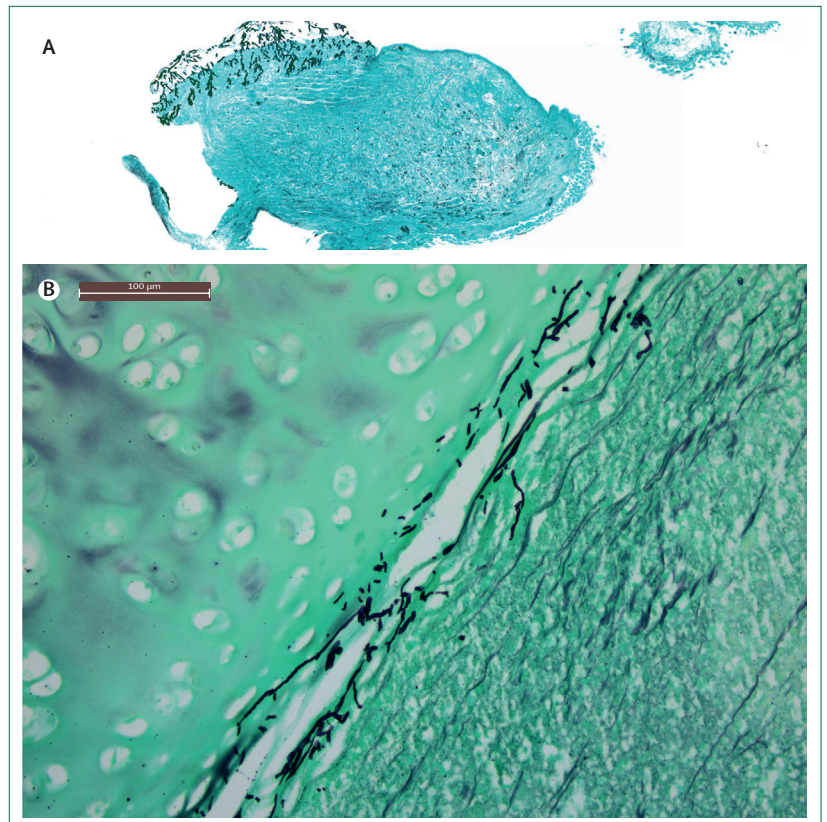


Figure 1: Biopsy images with Grocott stain from two patients with COVID-19 and invasive *Aspergillus* tracheobronchitis
(A) Bronchial biopsy showing an ulcer with necroinflammatory debris and septate *Aspergillus* hyphae (black; original magnification $\times 40$). Hyphae are superficially located and there is no evidence for angioinvasion. (B) Image of the trachea showing tissue invasion by fungal hyphae (black), consistent with *Aspergillus* (original magnification $\times 200$). There is no evidence for angioinvasion.

cells and the surrounding extracellular space, supporting a direct role of SARS-CoV-2 in the observed tissue damage.²¹ Furthermore, SARS-CoV-2 RNA has been detected in tracheal tissue.^{21,22} In the two patients with COVID-19 that were previously described (figures 1A, 1B), epithelial cells from the trachea and bronchi stained positive for ACE2 receptors, but the SARS-CoV-2 spike protein was not detected, possibly due to postmortem autolysis or the long interval between initial SARS-CoV-2 infection and autopsy. In an autopsy study of 38 people with COVID-19, large airway inflammation was noted in 35 (92%) patients, including acute inflammation in 17 patients and chronic inflammation in 18 patients.²⁰

Role of the host defence

The lytic infection that is caused by influenza and SARS-CoV-2 viruses results in a local innate immunodeficiency due to loss of the mechanical barrier function and capacity to produce defensins by the epithelial cells. Influenza has been observed to cause more extensive lysis compared with SARS-CoV-2,²³ which might have a role in the severity of the tracheobronchitis

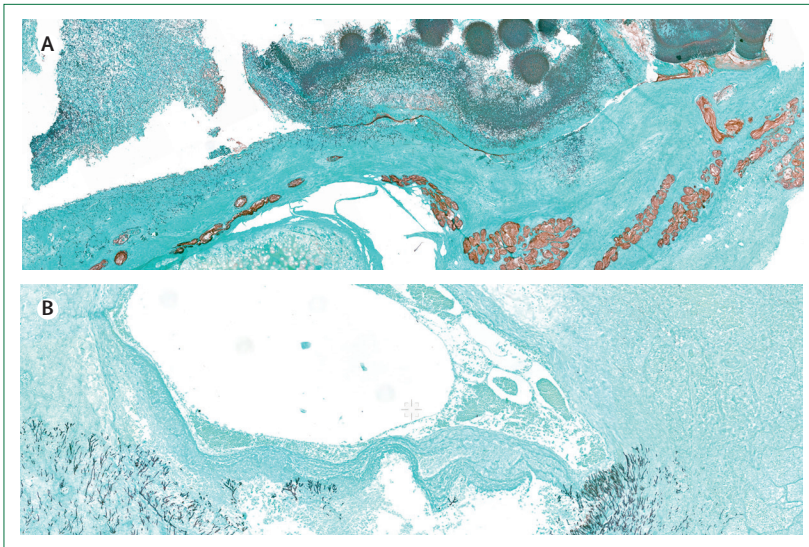


Figure 2: Histopathology of invasive *Aspergillus* tracheobronchitis in a patient with severe influenza shown with Grocott stain

(A) Trachea showing extensive ulceration and necrosis with presence of multiple *Aspergillus* hyphae (black; original magnification $\times 40$). The hyphae infiltrate the submucosa of the trachea. (B) *Aspergillus* hyphae (black) showing invasion of a tracheal artery vessel with thrombus formation (original magnification $\times 100$).

that is associated with influenza. Both COVID-19 and influenza share signs of hyperinflammation, which in some patients has features of macrophage activation syndrome, characterised by haemophagocytosis (among other features), defects in immune cell numbers (ie, pancytopenia), and increased ferritin concentration.^{24–27} This severe innate inflammatory response could have a role in tissue damage and necrosis at the site of infection, increasing the risk for invasive aspergillosis from the trachea. An impaired adaptive immune response reflected by lymphopenia is also shared by patients with COVID-19 and patients with influenza and is a well known risk factor for invasive aspergillosis. The severity of hyperinflammation and lymphopenia can vary in patients with COVID-19 or influenza, but both risk factors for aspergillosis are often present. Steroids are an independent risk factor in patients with IAPA; they have been associated with CAPA and can impair the ability of monocytes and macrophages to kill fungi²⁸ but can reduce the detrimental effects that are caused by cytokine storm syndrome. In contrast to influenza pneumonia, for which the use of steroids is not recommended due to the increased risk of invasive aspergillosis, steroids are now used as standard of care for patients with severe COVID-19 since publication of the data from the RECOVERY trial.²⁹ A difference between influenza and SARS-CoV-2 is that influenza can infect monocytes and macrophages^{30,31} and can impair the NADPH-oxidase in these cells.³² Patients with a genetic deficiency in NADPH-oxidase have an extreme predisposition to invasive aspergillosis,³³ and thus patients with influenza have a specific additional risk factor that predisposes them to severe invasive disease compared with patients with SARS-CoV-2. Another potential factor

that makes patients with influenza more susceptible to severe invasive aspergillosis than are patients with SARS-CoV-2 is the use of oseltamivir, which has been shown to impair fungal killing by myeloid cells.³⁴ The role of genetic factors that predispose a person to invasive disease in influenza and COVID-19 is unknown, but these factors are likely to contribute to the severity of disease.³⁵

Role of the fungus

A fumigatus has been the main species reported to cause invasive aspergillosis in association with influenza and COVID-19, but other *Aspergillus* species can cause IAPA and CAPA. *Aspergillus* species that can be involved in CAPA include *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, and *Aspergillus calidoustus*, but cases of confirmed IATB due to species other than *A fumigatus* have not yet been reported.³⁶ Although virulence traits have been identified in *A fumigatus*, which enable the fungus to better resist environmental stress factors, there is no evidence to support that specific *Aspergillus* genotypes cause invasive aspergillosis or IATB.¹ More than the fungus itself, the effects of the virus infection (eg, local cell lysis and subsequent effects on the host, including NADPH oxidase complex suppression in influenza) that are caused by the initial viral infection are likely to determine the ability of the host to resist fungal invasion. The histopathology shows distinct differences between IATB in critically ill patients with COVID-19 compared with patients with severe influenza. IATB in patients with severe influenza is characterised by a high fungal burden, deep tissue invasion, and angioinvasion when compared with patients with COVID-19. Additionally, in patients with influenza there is evidence for intraluminal sporulation of *A fumigatus* in the endotracheal plaques. Endotracheal production and release of *Aspergillus* conidia can increase the fungal burden in the lung. Furthermore, the presence of *Aspergillus* in the trachea and bronchial lumen reduces the options for sufficient drug exposure through systemic antifungal therapy. Clinically, IATB in patients with severe influenza is characterised by rapid progressive infection, and a study indicated that up to 70% (15 of 21) of patients with influenza had developed IAPA on admission to the ICU.³⁷ The course of disease in patients with IAPA seems to be less acute than for patients with CAPA, with a median time to CAPA diagnosis of 6–7 days after ICU admission, but specific data for IATB are scarce.

Angioinvasion threshold model

Insights into the histopathology of IATB and observations from cohort studies of patients with IAPA and CAPA suggest that the interplay between the host, virus, and fungus are crucial determinants of clinical disease progression and subsequently diagnostic test performance (figure 3). Angioinvasion is a central feature of the pathogenesis of invasive aspergillosis,³⁸ leading to intravascular thrombosis and tissue infarction

that causes tissue necrosis and reduced entry of leukocytes.³⁹ Furthermore, angioinvasion is an important condition for components of the fungal cell wall, such as galactomannan, to be released into the circulation. In patients with influenza, IATB was diagnosed at a median of 2 days after ICU admission (range 1–5 days), and circulating galactomannan was detected in 89% (8 of 9) of these patients.¹⁴ These observations indicate that angioinvasion occurs early in the disease process. Compared with SARS-CoV-2 infection, more severe and extensive influenza-induced epithelial damage and impaired NADPH-oxidase reactivity due to infection of monocytes and macrophages can enable the fungus to reach the threshold for angioinvasion early in the disease process. The ability to reach the angioinvasion threshold can be further accelerated by treatment with neuraminidase inhibitors, ultimately leading to the higher fungal burden that is observed in patients with influenza compared with COVID-19. However, although specific data for IATB in patients with COVID-19 are still scarce, CAPA is often diagnosed later after ICU admission than is IATB in patients with IAPA, and circulating galactomannan is rarely detected.^{8,9} Repeated testing of serum samples can increase the sensitivity of the galactomannan assay. Some centres did routine galactomannan serum screening in patients with COVID-19,¹⁰ including our own centre, whereas others have not done serum testing on a routine basis.⁸ However, low serum galactomannan positivity is consistently reported in patients with CAPA, suggesting low sensitivity rather than testing of an insufficient number of samples. These clinical observations are in line with the absence of angioinvasion in the histopathology, even after corticosteroid therapy and extended ICU stay. Detection of circulating galactomannan can be a marker for more advanced disease in critically ill patients with COVID-19, as indicated in a study where the ICU mortality in patients with proven or probable CAPA who were serum positive for galactomannan was 88% (7 of 8) compared with 37% (7 of 19) in patients who were serum negative for galactomannan (odds ratio 10.000 [95% CI 1.030–97.044]; $p=0.047$; Verweij PE, unpublished). The absence of serum galactomannan positivity in patients with CAPA contrasts with the high prevalence of galactomannan-positive serum in patients with influenza, suggesting that the ability to develop angioinvasion differs for patients with CAPA compared with IAPA. The specific aggregation of predisposing factors (eg, presence of EORTC and MSGERC factors or genetic polymorphisms), viral factors (eg, tropism and lytic effects), host defence factors, and effects of antiviral or immunosuppressive therapies will determine whether the angioinvasion threshold is reached and when (figure 3).

Management of IATB

Interventions to prevent rapid *Aspergillus* airway disease progression should target all four determinants that have been described: predisposing factors, virus infection,

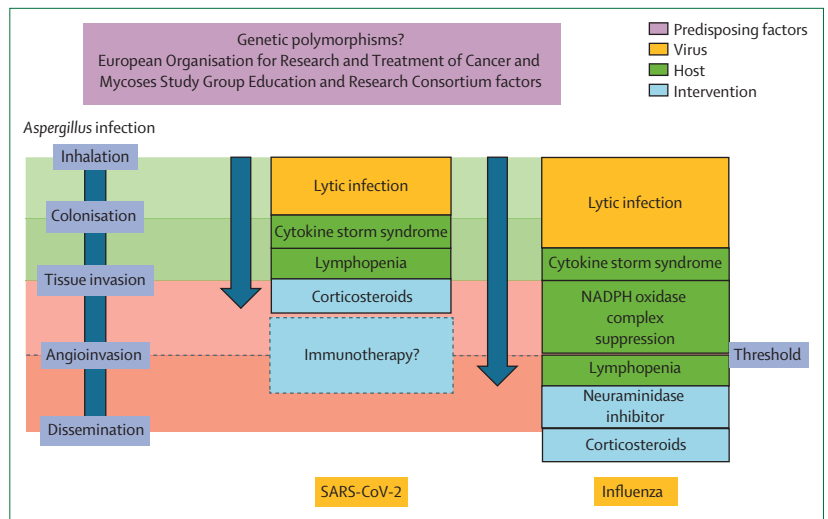


Figure 3: The angioinvasion threshold model
Factors that contribute to invasive *Aspergillus* tracheobronchitis disease progression ultimately leading to angioinvasion in patients with severe COVID-19 and influenza pneumonia. The arrows indicate invasive *Aspergillus* tracheobronchitis disease progression, where the infection has surpassed the angioinvasion threshold in a case of influenza.

host factors, and interventions (figure 4). Genetic polymorphisms and EORTC and MSGERC host factors inevitably contribute to the risk and clinical course of CAPA and IAPA. These aspects should be considered as an a priori risk for developing disease and cannot be modified.

Beyond these predisposing factors, the following strategies can contribute to managing IATB: prevention or reduction of severity of lytic infection with strategies that are targeted at the virus, host-directed strategies that are aimed at balancing immune dysregulation, and targeted anti-*Aspergillus* therapy. Disease-specific interventions are depicted in figure 4 and are based on the pathophysiology of IATB, which is different between patients with severe COVID-19 and patients with influenza. These strategies targeting the virus, host, and fungus can be deployed as individual interventions but most likely will be combined. Interventions might exert a beneficial effect on one factor but create a risk for another factor (figure 4). For example, the neuraminidase inhibitor oseltamivir might reduce the lytic effects that are caused by influenza but might predispose the patient to invasive aspergillosis via effects on host neuraminidase activity.³⁴ Similarly, corticosteroids might exert a beneficial effect on reducing the cytokine storm syndrome, but their use has also irrevocably been associated with increased risk of IAPA and CAPA.^{2,8}

Rebalancing the immune dysregulation can be done by decreasing the cytokine storm syndrome with anti-inflammatory strategies (eg, anti-interleukin-1 or anti-interleukin-6 biological therapies)⁴⁰ or restoring immune paralysis by agents, such as recombinant interferon gamma.⁴¹ The timing of these treatments in patients with influenza or COVID-19 is pivotal. Generally,

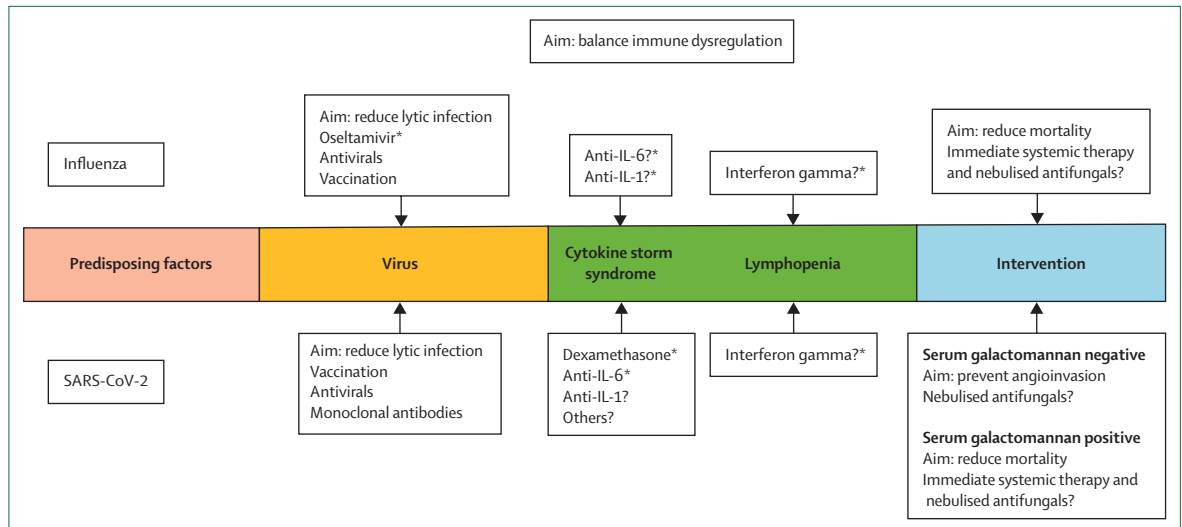


Figure 4: Interventions that might contribute to reducing the severity and mortality associated with invasive *Aspergillus tracheobronchitis*
 IL=interleukin. *Can have both beneficial and harmful effects on reaching threshold.

a hyperinflammatory status will be recognised early in the presentation of severe disease, whereas immune paralysis often occurs later in disease. Notably, these two conditions can coexist. These interventions will benefit all manifestations of CAPA and IAPA, but the management of IATB presents several additional challenges, especially in patients with severe influenza. Given the high mortality and rapid onset of disease in critically ill patients with influenza, immediate bronchoscopy and biopsy of visible plaques is indicated. Considering the rapid onset with early angioinvasion and high fungal burden, an aggressive therapeutic approach involving systemic antifungal therapy is justified. Yet, if plaques have formed in the trachea, systemic drugs alone might not be sufficient, as antifungal drug penetration is expected to be low and bronchi are poorly penetrable. In such situations, adding nebulised antifungals to systemic therapy might be of value, although clinical evidence supporting nebulised therapy is scarce.⁴²

Contrary to IAPA, IATB in critically ill patients with COVID-19 presents as a less acute disease. Mucosal biopsy of plaques will discriminate between airway colonisation and invasive infection by showing tissue invasive growth of septae hyphae in cases of invasive infection, but it is unclear how IATB in patients with COVID-19 is best managed. Initiating systemic antifungal therapy will depend on the presence of other manifestations of CAPA (as shown by positive bronchoalveolar lavage galactomannan), whereas in patients with negative bronchoalveolar lavage galactomannan there might be a role for (pre-emptive) nebulised antifungal therapy. Serum galactomannan could serve as the biomarker of choice to show presence of angioinvasion, and immediate systemic antifungal therapy would be required given the high mortality rate that is reported in patients with CAPA who are serum

galactomannan positive. Prevention of CAPA and IATB through chemoprophylaxis might be an alternative approach, but there are no antifungal agents that are licensed for prophylactic use in the ICU. Such a strategy would require selection of patients in ICU who are at high risk of developing CAPA and IATB based on predisposing factors, such as chronic lung disease or EORTC and MSGERC host factors, to avert overuse of antifungal agents. Patients with visible plaques or a positive bronchial aspirate culture, but negative bronchoalveolar lavage galactomannan, might benefit from pre-emptive administration of local or systemic antifungals.

Conclusions and future directions

Current understanding of the pathophysiology of IATB in patients with COVID-19 and patients with influenza has allowed us to hypothesise that the threshold for the indication to start systemic antifungal therapy coincides with the presence or absence of angioinvasion. Whether this hypothesis is true for all patients with IAPA and CAPA is unknown: not all patients present with a clinical picture of tracheobronchitis, and patients with tracheobronchitis can still have signs of invasive pulmonary disease (shown by an elevated bronchoalveolar lavage galactomannan) that warrant systemic treatment. Although antifungal therapy is recommended for patients with possible, probable, or proven CAPA,¹² several studies observed that patients who were diagnosed with CAPA (based on bronchoalveolar lavage positivity) survived without receiving antifungal therapy,^{6,8} most likely because the threshold of angioinvasion was not reached and the risk factors, such as the virus itself, cytokine storm syndrome, and lymphopenia, were resolved. The progression from *Aspergillus* colonisation to tissue invasion and angio-

Search strategy and selection criteria

We identified references for this Personal View through searches of PubMed for articles published from January, 1971, to January, 2021, with the terms "COVID-19", "coronavirus", "influenza", "aspergillosis", "tracheobronchitis", "IAPA", and "CAPA". We reviewed the articles that resulted from the searches and relevant references that were cited in the initial articles. We excluded articles that were not published in English.

invasion is probably a continuum, and diagnostic tests that are available do not have the ability to be stage specific. Identification of biomarkers to indicate tissue invasion stages and airway involvement should be a priority of future studies. Furthermore, the described threshold suggests that applying various antiviral, immunomodulatory, and antifungal drugs (eg, nebulised antifungals) can help to prevent progression from *Aspergillus* colonisation to tissue invasion and angioinvasion.

The main challenge for future research is the design of clinical trials that take into consideration the complexity of a combined intervention on these three levels (ie, antiviral, immunomodulatory, and antifungal) of influence on the threshold for angioinvasive disease. Ideally, the intervention arms are guided by biomarkers that are usable in daily clinical practice, resulting in a true example of a personalised trial. Unfortunately, there are no clear data yet that cytokines or ratio of cytokines can guide immunotherapy. Most guidance is now based on clinically used parameters. Identification of the prevailing immune status of the patient is important for guiding personalised immunotherapy. Thus, patients with an immune status that is characterised by hyperinflammation would most likely benefit from anti-inflammatory therapy (eg, anticytokine biologicals) that would reduce tissue damage. By contrast, in patients in whom immune paralysis is prevailing, adjuvant stimulatory immunotherapy (eg, recombinant interferon gamma) is likely to be beneficial. Future studies with personalised immunotherapy approaches should be a priority for identifying the best therapeutic approaches in patients with CAPA and IAPA, both with and without IATB.

Contributors

FLvdV, PEV, RJMB, and MGN conceptualised this Personal View. SV and GDH investigated the histology. JW, FLvdV, JAS, and MHER investigated the clinical management. PEV, FLvdV, and RJMB wrote the first draft. SV, GDH, JW, MHER, MGN, and JAS contributed to writing and editing.

Declaration of interests

RJMB reports grants from and consultancy for MSD, Pfizer, Mundipharma, F2G, Gilead Sciences, Astellas Pharma, and Amplyx Pharmaceuticals, outside of the submitted work. JW reports grants and personal fees from MSD, Gilead Sciences, and Pfizer during the study. PEV reports grants from Mundipharma, F2G, Pfizer, Thermo Fisher Scientific, Gilead Sciences, and Cidara Therapeutics and non-financial support from IMMY, outside of the submitted work. FLvdV reports

personal fees from Gilead Sciences and Swedish Orphan Biovitrum, outside of the submitted work. SV, GDH, MHER, MGN, and JAS declare no competing interests.

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