



Invasive pulmonary aspergillosis associated with viral pneumonitis

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The occurrence of invasive pulmonary aspergillosis (IPA) in critically ill patients with viral pneumonitis has increasingly been reported in recent years. Influenza-associated pulmonary aspergillosis (IAPA) and COVID-19-associated pulmonary aspergillosis (CAPA) are the two most common forms of this fungal infection. These diseases cause high mortality in patients, most of whom were previously immunocompetent. The pathogenesis of IAPA and CAPA is still not fully understood, but involves viral, fungal and host factors. In this article, we discuss several aspects regarding IAPA and CAPA, including their possible pathogenesis, the use of immunotherapy, and future challenges.

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Introduction

Invasive pulmonary aspergillosis (IPA), caused by the ubiquitous fungus *Aspergillus*, characteristically occurs in immunocompromised individuals, notably those with prolonged neutropenia, recipients of hematopoietic stem cell

or solid organ transplants, or patients with hematological malignancies [1–4]. Recent outbreaks of respiratory viral diseases such as severe acute respiratory syndrome (SARS), H5N1 avian flu, the 2009 H1N1 influenza pandemic, and recently coronavirus disease 2019 (COVID-19), caused by severe respiratory syndrome coronavirus 2 (SARS-CoV-2) have underlined the importance of respiratory viruses as an important cause of severe pneumonia in adults [5]. In recent years, IPA has been described increasingly in these patients [1,6,7,8*,9,10*,11–17,18*]. IPA affects critically ill patients with viral pneumonia due to two viruses in particular: influenza and SARS-CoV-2.

Influenza-associated pulmonary aspergillosis (IAPA) occurs in critically ill patients in the intensive care unit (ICU) with severe influenza (predominantly influenza A), and is associated with a high ICU mortality of 45–61% compared to 20% in ICU patients with influenza without IAPA [1,8*,9,19]. Importantly, underlying classical risk factors for IPA are present in approximately only half of patients who develop IAPA [8*,9]. Furthermore, increasing acute physiology and chronic health evaluation (APACHE) II score at admission was an independent risk factor for IAPA in one study, indicating an association between influenza severity and risk of IPA [8*]. Besides IAPA, invasive aspergillosis has also been increasingly reported in patients with COVID-19 [10*,17,20]. The incidence of COVID-19-associated pulmonary aspergillosis (CAPA) among patients with COVID-19 is approximately 4–33%, and mortality is 44–71%, higher compared to patients with COVID-19 without CAPA (19–37%) [15–17]. Although IAPA and CAPA share certain clinical characteristics, such as the frequent absence of classical risk factors as defined by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC), absence of typical radiographic features and a high associated mortality, there are distinct differences as well, for example in terms of time of development and association with the use of corticosteroids. Whereas IAPA tends to develop early in the course of ICU admission (at a median of three days after ICU admission) [8*,9], CAPA develops after a median of 4–8 days after ICU admission or intubation [15,16]. Furthermore, whereas corticosteroids have been shown to constitute a clear risk factor for the development of IAPA, their role in CAPA risk varies between studies [8*,15–17].

IPA incidence in patients with influenza or COVID-19 in ICU is evidently higher than that in patients admitted to ICU with acute respiratory distress syndrome (ARDS) due to any cause (4%) [21], with community-acquired pneumonia (5%) [8] or with severe respiratory syncytial virus (RSV) pneumonia (3.5%) [7]. This suggests a specific role of the causative viral agent in the increased susceptibility to IPA. In this review, we will elaborate on the possible pathogenesis of these two most common forms of IPA complicating viral pneumonitis, as well as possible and available adjunctive immune-based therapies.

Pathogenesis of IAPA and CAPA

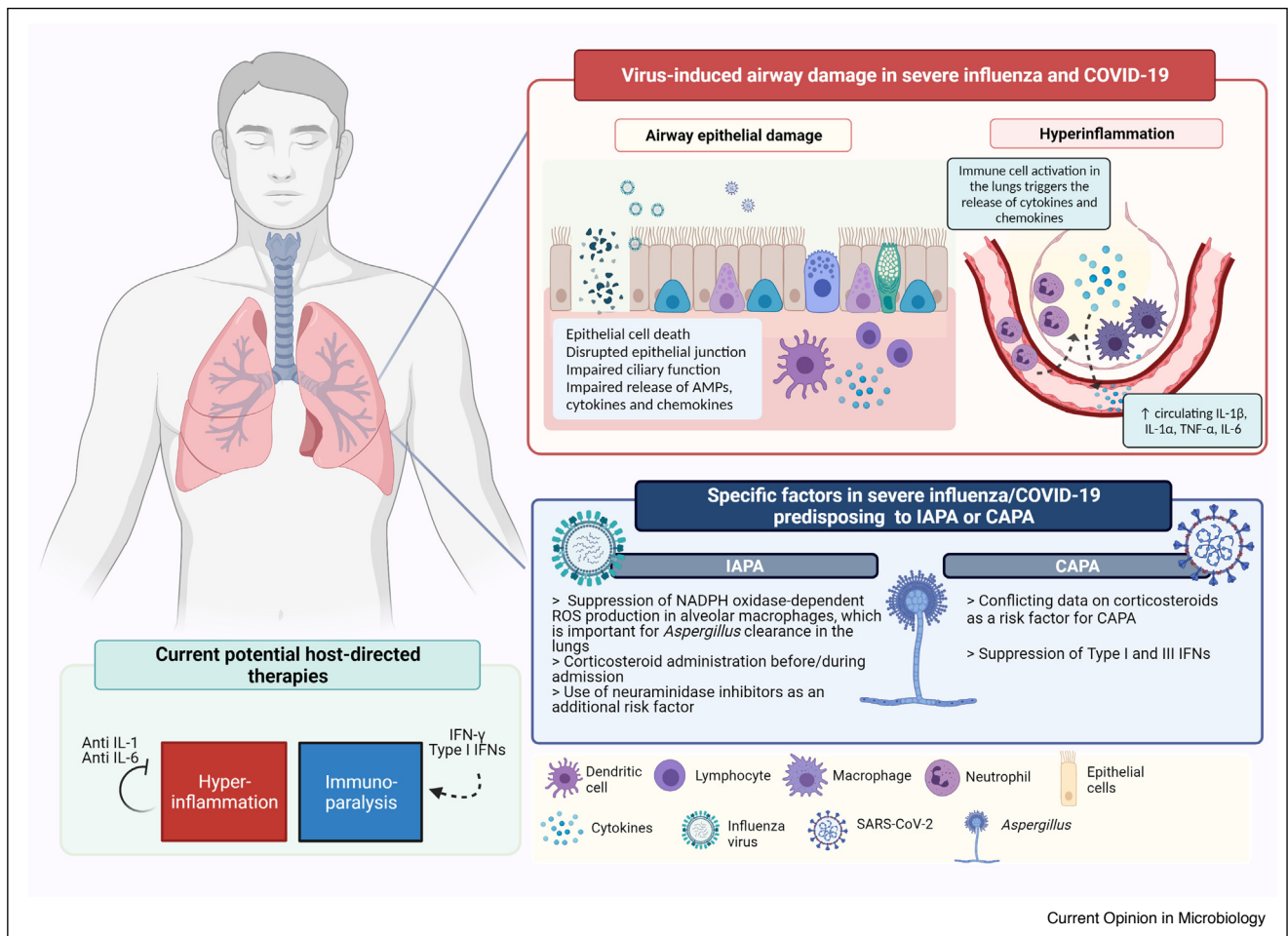
The pathogenesis of IAPA and CAPA is still not fully understood, but includes multiple factors caused by the viral infection, *Aspergillus* itself, and the host immune response. Influenza virus differs from SARS-CoV-2 in terms of cell tropism and viral replicative properties [22,23]. Influenza binds to sialic acids on respiratory epithelial cell surfaces via viral hemagglutinin (HA), providing cellular entry for replication. New virions are released only after cleavage of their connection with the host cell via sialic acids (SAs) by viral neuraminidase. Human adapted influenza virus strains preferably bind α -2,6-linked SAs, which are mainly present in the upper airways [24]. In contrast, avian influenza strains preferably bind α -2,3-linked SAs, which are expressed in birds' intestinal tracts, but also in the human lower respiratory tract. Therefore, increased HA binding affinity for α -2,3-linked SAs in human influenza strains (as in the pandemic H1N1 strain and the avian influenza H5N1 strain), and dual receptor specificity (as in the H7N9 avian influenza strain) are hypothesized to contribute to increased pathogenicity. However, receptor binding specificity is not the only determinant for pathogenicity [25,26]. Furthermore, although IAPA has predominantly been described in patients with influenza A or specific influenza A strains (H1N1, H3N2, H7N9) [1,8,9,27,28], no direct associations between IAPA risk and specific influenza strains have been described thus far. Influenza's lytic effects on tracheobronchial epithelial cells may provide a portal of entry for *Aspergillus* to cause tissue invasion, which clinically presents as invasive *Aspergillus* tracheobronchitis. SARS-CoV-2, however, targets cells expressing angiotensin converting enzyme 2 (ACE2) and the transmembrane protease serine 2 (TMPRSS2), which facilitate its binding and entry [29]. These include airway epithelial cells, type 2 pneumocytes, vascular endothelial cells, and alveolar macrophages [30–33]. Although invasive *Aspergillus* tracheobronchitis has been described in both IAPA and CAPA, the reported frequencies in patients with severe influenza are higher compared to those with COVID-19 [18].

Despite the differences in host cell-binding specificity, both influenza and SARS-CoV-2 cause airway epithelial

damage, usually characterized by disrupted epithelial junctions, impaired ciliary clearance, and loss of cell functions, such as the release of antimicrobial proteins (Figure 1) [34,35]. In the lungs, virus-infected cells release danger-associated molecular patterns (DAMPs), which are detected by adjacent epithelial cells and resident alveolar macrophages. Subsequent activation of these cells results in the release of proinflammatory cytokines, including interleukin (IL)-6, interferon (IFN)- γ , IL-1 β , and tumor necrosis factor (TNF)- α , and various chemokines. These immune mediators trigger influx of macrophages and neutrophils, further aggravating the local inflammatory response [36–39]. Influenza (strain A/PR/8/34; H1N1) has also been shown to induce apoptosis of alveolar macrophages, contributing to the increased susceptibility to secondary pneumococcal infection [40]. Hyperinflammation is a common feature of both influenza and COVID-19, characterized by high concentrations of circulating inflammatory cytokines, acute phase reactants and ferritin, and hemophagocytosis, some of which are typical features of macrophage activation syndrome (MAS) [41,42]. Lymphopenia is also observed in both influenza and COVID-19 [43,44]. The decreased CD4⁺ and CD8⁺ lymphocyte populations are often accompanied by impaired T cell mediated responses in the latter [45].

Host defense against *Aspergillus* involves multiple immune system components. The first important layer is the airway epithelium. The release of various antimicrobial proteins, cytokines and chemokines by airway epithelial cells is essential for initial defense against *Aspergillus* [46,47]. Since the integrity of innate immune barriers in the airways is crucial to prevent invasive growth of *Aspergillus* into the pulmonary parenchyma, it is likely that airway damage induced by influenza and SARS-CoV-2 predisposes patients to viral pneumonitis-associated IPA. However, since ARDS in itself also causes loss of epithelial cell integrity, but is associated with a much lower incidence of IPA [21], other factors associated with the viral infection and antiviral immune response must be involved in susceptibility to viral pneumonitis-associated IPA. The second crucial pulmonary anti-*Aspergillus* defense layer involves alveolar macrophages and neutrophils [48,49]. These cells eliminate *Aspergillus* conidia by phagocytosis and release of reactive oxygen species (ROS), as well as by production of cytokines and chemokines [50–52]. Although ROS have been implicated in (X-31/H3N2) influenza-induced lung injury [53], influenza virus (strain A/PR/8) has been shown to suppress NADPH-oxidase-dependent ROS production in alveolar macrophages and neutrophils in an animal model, affecting their bactericidal capacity [54]. This transient suppression of ROS production mimics the phenotype of chronic granulomatous disease (CGD). CGD patients are genetically deficient in one of the components of the NADPH oxidase complex type

Figure 1



Proposed pathogenesis of IAPA and CAPA.

The pathogenesis of IAPA and CAPA share several common features, including the presence of airway epithelial damage and hyperinflammation (top right panel). However, different factors specific for influenza and COVID-19 might further underlie the increased susceptibility to IPA in these viral infections (bottom right panel). Potential host-directed therapies are highlighted in brief (bottom left panel). Figure created with [Biorender.com](https://www.biorender.com) (AMP, Antimicrobial protein. CAPA, COVID-19-associated pulmonary aspergillosis. COVID-19, Coronavirus disease 2019. IAPA, Influenza-associated pulmonary aspergillosis. IFN, Interferon. IL, Interleukin. IPA, Invasive pulmonary aspergillosis. NADPH, Nicotinamide adenine dinucleotide phosphate. ROS, Reactive oxygen species. TNF- α , tumor necrosis factor- α).

2 and are highly susceptible to invasive aspergillosis [55]. Furthermore, ROS deficiency in these patients leads to defective formation of a noncanonical autophagosome in macrophages, a process known as LC3-associated phagocytosis (LAP) [56]. LAP is crucial for *Aspergillus* elimination in phagocytic cells, whereby *Aspergillus*-containing phagosomes are efficiently targeted for lysosomal degradation [57,58]. Additionally, corticosteroids have been shown to inhibit recruitment of the LC3II protein, a crucial step in LAP [59]. Based on these findings, one could speculate that ROS suppression by influenza might lead to impaired LAP, resulting in uninhibited growth of *Aspergillus*, an effect worsened by use of corticosteroids.

Recent studies have explored the differences in immunological characteristics between patients with severe influenza and COVID-19. An important example is the decreased type I and III IFN response in COVID-19 patients [60[•],61]. Bastard *et al.* described the presence of neutralizing autoantibodies against type I IFNs in approximately 10% of patients with life-threatening COVID-19. These autoantibodies could negate these type I IFNs' ability to block SARS-CoV2 infection *in vitro* [62[•]]. Type I IFNs are known to be key drivers of type III IFN (IFN- λ) production, which stimulates neutrophil actions against *Aspergillus fumigatus* [63]. Furthermore, addition of IFN- β to human dendritic cells is able to promote anti-*Aspergillus* T helper type 1 responses [64].

Whether a diminished type I IFN response in COVID-19 is associated with an increased susceptibility to *Aspergillus* infection is not yet known.

Another differential contributor to the pathogenesis of IAPA and CAPA could be the use of neuraminidase inhibitors. These agents are administered in patients with severe influenza to limit viral replication [65]. The possible role of neuraminidase inhibitors in the pathogenesis of IAPA has been studied by our group. We have described that neuraminidase is crucial for anti-*Aspergillus* defense *in vitro*, and that inhibition of endogenous neuraminidase by oseltamivir impairs the capacity of human monocytes and murine splenocytes to kill *Aspergillus* [66]. Additionally, oseltamivir-treated immunocompetent mice exhibited higher fungal burdens in the lungs and higher mortality after infection with *Aspergillus* compared with untreated controls. Therefore, although the use of neuraminidase inhibitors could prevent worsening of viral replication in patients with severe influenza, prolonged use might increase susceptibility to *Aspergillus* infection.

Implications of the pathogenesis for the clinical presentation of IAPA and CAPA

The abovementioned factors have important consequences for the clinical presentation and management of IAPA and CAPA. The combination of the lytic effects of influenza, inhibition of ROS production, immune dysregulation and presence of predisposing factors, such as EORTC/MSGERC host factors, result in a host highly susceptible to IPA. As a consequence, IAPA is frequent, occurs early after ICU admission and is a rapidly progressive infection. Furthermore, in patients with invasive *Aspergillus* tracheobronchitis a mortality of 90% was reported [67^{**}]. The high proportion of patients with positive serum galactomannan (GM), that is, 90% in influenza patients with invasive *Aspergillus* tracheobronchitis and 65% in IAPA patients, indicates that angioinvasive growth occurs early in the disease process.

The course of infection is different in patients with CAPA, which may be due to less severe lytic effects of SARS-CoV-2 on respiratory epithelium and lack of (known) viral effects on fungal host defense pathways. However, immune dysregulation and possible EORTC/MSGERC host factors do contribute to susceptibility to IPA. The infection occurs at a median of seven days after ICU admission, indicating a less acute disease progression compared with IAPA. This is supported by the low frequency of serum GM detection in 15% of CAPA patients and lack of angioinvasion, as opposed to tissue invasion, in histopathological specimens of infected patients. Nevertheless, the CAPA ICU mortality rates of 52% are similar to those observed in IAPA [10^{*}].

Immunotherapy: rebalancing the host response

The optimal management of viral pneumonitis-associated aspergillosis should not only include antiviral and antifungal treatments, but also a consideration of adjunctive host-directed therapy aimed at resolving the dysregulation of the immune response. Immunopathology in IAPA and CAPA is not only due to *Aspergillus*-induced inflammation, but also to collateral damage resulting from a dysregulated immune response against the fungus and direct effects of the virus. On the one hand, a strong immune response is important for effective pulmonary clearance of *Aspergillus*. On the other hand, excessive inflammation might induce tissue damage, and thus impair pulmonary function. In this regard, the timing and type of treatment are crucial to achieve a balanced immune response.

Immunomodulatory therapies aimed at reducing systemic inflammation have been explored in COVID-19 (Figure 1). Tocilizumab, an anti-IL-6 drug, improves clinical outcome in a subset of patients with COVID-19, but is not effective in all patients, particularly those with moderate to severe disease [68,69]. However, secondary infections as a possible immunological consequence of IL-6 blockade should be kept in mind, although current evidence does not strongly support this [70]. Furthermore, inhibition of the IL-1 receptor with anakinra has been shown to be beneficial in COVID-19 patients, resulting in ameliorated clinical symptoms and reduced inflammatory parameters [71,72^{*},73,74]. The role of corticosteroid use in viral pneumonitis is more complex. Corticosteroids are a well-known risk factor for the development of IPA. In ICU patients with influenza, corticosteroid use is associated with higher mortality [75]; in addition, it is independently associated with the development of IAPA [8^{*}]. However, in COVID-19, dexamethasone has been shown to reduce mortality of patients receiving ventilatory support [76^{*}]. In contrast to IAPA, literature on the effects of corticosteroid use on CAPA risk and outcome is conflicting. Apart from immunosuppressive agents, administration of immunostimulatory agents should also be considered during the course of COVID-19. In later disease stages with immune paralysis often present, administration of recombinant IFN- γ or type I IFNs could be beneficial [77]. IFN- γ has been shown to restore immune functions in patients with fungal sepsis, notably by improving antigen presentation and the capacity of immune cells to produce proinflammatory cytokines [78].

Summary and future perspectives

Viral pneumonitis is increasingly recognized as a risk factor for IPA, and the fact that the majority of cases occur in previously immunocompetent individuals is concerning. Understanding the pathogenesis of viral pneumonitis-associated IPA from a perspective of the virus,

the fungus, and the host, preferably in an integrated model system is imperative [79]. Mortality rates of IAPA and CAPA are high despite antifungal therapy, indicating that an integrated approach involving interventions aimed at diminishing the effects of the viral infection, early diagnosis and treatment of the fungal infection and rebalancing of the immune dysregulation, may improve patient outcome. Early screening for *Aspergillus* infection in critically ill patients with influenza is crucial in limiting disease progression, and administration of antifungal agents immediately following ICU admission of patients with severe influenza might be indicated in regions with high IAPA incidence. Given the variation in immune dysregulation, that is, hyperinflammation or immune paralysis, determining the prevailing immune status of the patient is critical in order to select the appropriate host-directed intervention. In this case, measurement of circulating immune markers at several time points could be a valuable approach. Integrated and personalized treatment strategies need to be incorporated into future clinical trials in order to determine which approaches are successful in reducing IAPA and CAPA mortality.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. van de Veerdonk FL, Kolwijck E, Lestrade PP, Hodiament CJ, Rijnders BJ, van Paassen J et al.: **Influenza-associated aspergillosis in critically ill patients.** *Am J Respir Crit Care Med* 2017, **196**:524-527.
2. Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ et al.: **Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) database.** *Clin Infect Dis* 2010, **50**:1091-1100.
3. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A et al.: **Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET).** *Clin Infect Dis* 2010, **50**:1101-1111.
4. Mühlemann K, Wenger C, Zenhäusern R, Täuber MG: **Risk factors for invasive aspergillosis in neutropenic patients with hematologic malignancies.** *Leukemia* 2005, **19**:545-550.
5. Ruuskanen O, Lahti E, Jennings LC, Murdoch DR: **Viral pneumonia.** *Lancet* 2011, **377**:1264-1275.
6. Helleberg M, Steensen M, Arendrup MC: **Invasive aspergillosis in patients with severe COVID-19 pneumonia.** *Clin Microbiol Infect* 2021, **27**:147-148.
7. Nam H, Ison MG: **1496. Aspergillosis complicating severe respiratory syncytial virus (RSV) in ICU patients: a retrospective cohort study.** *Open Forum Infect Dis* 2020, **7** (Suppl. 1):S750.
8. Schauwvlieghe A, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C et al.: **Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study.** *Lancet Respir Med* 2018, **6**:782-792.
- This study identifies influenza as an independent risk factor for influenza-associated pulmonary aspergillosis (IAPA).
9. Wauters J, Baar I, Meersseman P, Meersseman W, Dams K, De Paep R et al.: **Invasive pulmonary aspergillosis is a frequent complication of critically ill H1N1 patients: a retrospective study.** *Intensive Care Med* 2012, **38**:1761-1768.
10. Koehler P, Cornely OA, Böttiger BW, Dusse F, Eichenauer DA, Fuchs F et al.: **COVID-19 associated pulmonary aspergillosis.** *Mycoses* 2020, **63**:528-534.
- This study reports the occurrence of COVID19-associated pulmonary aspergillosis in critically ill patients with acute respiratory distress syndrome (ARDS).
11. Rutsaert L, Steinfert N, Van Hunsel T, Bomans P, Naesens R, Mertes H et al.: **COVID-19-associated invasive pulmonary aspergillosis.** *Ann Intensive Care* 2020, **10**:71.
12. Wang J, Yang Q, Zhang P, Sheng J, Zhou J, Qu T: **Clinical characteristics of invasive pulmonary aspergillosis in patients with COVID-19 in Zhejiang, China: a retrospective case series.** *Crit Care* 2020, **24**:299.
13. Alanio A, Dellièvre S, Fodil S, Bretagne S, Mégarbane B: **Prevalence of putative invasive pulmonary aspergillosis in critically ill patients with COVID-19.** *Lancet Respir Med* 2020, **8**: e48-e49.
14. Lamothe F, Glampedakis E, Boillat-Blanco N, Oddo M, Pagani JL: **Incidence of invasive pulmonary aspergillosis among critically ill COVID-19 patients.** *Clin Microbiol Infect* 2020, **26**:1706-1708.
15. Bartoletti M, Pascale R, Cricca M, Rinaldi M, Maccaro A, Bussini L et al.: **Epidemiology of invasive pulmonary aspergillosis among COVID-19 intubated patients: a prospective study.** *Clin Infect Dis* 2020 <http://dx.doi.org/10.1093/cid/ciaa1065>. Online ahead of print.
16. White PL, Dhillion R, Cordey A, Hughes H, Faggian F, Soni S et al.: **A national strategy to diagnose COVID-19 associated invasive fungal disease in the ICU.** *Clin Infect Dis* 2020 <http://dx.doi.org/10.1093/cid/ciaa1298>. Online ahead of print.
17. Dellièvre S, Dudoignon E, Fodil S, Voicu S, Collet M, Ouilic PA et al.: **Risk factors associated with COVID-19-associated pulmonary aspergillosis in ICU patients: a French multicentric retrospective cohort.** *Clin Microbiol Infect* 2020 <http://dx.doi.org/10.1016/j.cmi.2020.12.005>. Online ahead of print.
18. van Arkel ALE, Rijpsma TA, Belderbos HNA, van Wijngaarden P, Verweij PE, Bentvelsen RG: **COVID-19-associated pulmonary aspergillosis.** *Am J Respir Crit Care Med* 2020, **202**:132-135.
- This study reports the incidence of CAPA in ICU patients with COVID-19 and did not observe invasive *Aspergillus* tracheobronchitis in any CAPA patient.
19. Crum-Cianflone NF: **Invasive aspergillosis associated with severe influenza infections.** *Open Forum Infect Dis* 2016, **3**: ofw171.
20. Borman AM, Palmer MD, Fraser M, Patterson Z, Mann C, Oliver D et al.: **COVID-19-associated invasive aspergillosis: data from the UK national mycology reference laboratory.** *J Clin Microbiol* 2020, **59**:e02136-20.
21. Contou D, Dorison M, Rosman J, Schlemmer F, Gibelin A, Foulet F et al.: **Aspergillus-positive lower respiratory tract samples in patients with the acute respiratory distress syndrome: a 10-year retrospective study.** *Ann Intensive Care* 2016, **6**:52.
22. Ibricevic A, Pekosz A, Walter MJ, Newby C, Battaile JT, Brown EG et al.: **Influenza virus receptor specificity and cell tropism in mouse and human airway epithelial cells.** *J Virol* 2006, **80**:7469-7480.

23. Benton DJ, Wrobel AG, Xu P, Roustan C, Martin SR, Rosenthal PB *et al.*: **Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion.** *Nature* 2020, **588**:327-330.
 24. Qi L, Kash JC, Dugan VG, Wang R, Jin G, Cunningham RE *et al.*: **Role of sialic acid binding specificity of the 1918 influenza virus hemagglutinin protein in virulence and pathogenesis for mice.** *J Virol* 2009, **83**:3754-3761.
 25. de Graaf M, Fouchier RA: **Role of receptor binding specificity in influenza A virus transmission and pathogenesis.** *EMBO J* 2014, **33**:823-841.
- This review provides an overview of the role of hemagglutinin and host sialic acids in the pathogenesis and transmission of influenza A virus.
26. Mair CM, Ludwig K, Herrmann A, Sieben C: **Receptor binding and pH stability - how influenza A virus hemagglutinin affects host-specific virus infection.** *Biochim Biophys Acta* 2014, **1838**:1153-1168.
- This review gives an overview of hemagglutinin receptor specificity and stability in influenza A infection.
27. Huang L, Zhang N, Huang X, Xiong S, Feng Y, Zhang Y, Li M, Zhan Q: **Invasive pulmonary aspergillosis in patients with influenza infection: a retrospective study and review of the literature.** *Clin Respir J* 2019, **13**:202-211.
 28. Zou P, Wang C, Zheng S, Guo F, Yang L, Zhang Y, Liu P, Shen Y, Wang Y, Zhang X *et al.*: **Invasive pulmonary aspergillosis in adults with avian influenza A (H7N9) pneumonia in China: a retrospective study.** *J Infect Dis* 2020, **221**:S193-S197.
 29. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S *et al.*: **SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor.** *Cell* 2020, **181**:271-280.e8.
 30. Lee IT, Nakayama T, Wu C-T, Goltsev Y, Jiang S, Gall PA *et al.*: **ACE2 localizes to the respiratory cilia and is not increased by ACE inhibitors or ARBs.** *Nat Commun* 2020, **11**:5453.
 31. Song X, Hu W, Yu H, Zhao L, Zhao Y, Zhao Y: **High expression of angiotensin-converting enzyme-2 (ACE2) on tissue macrophages that may be targeted by virus SARS-CoV-2 in COVID-19 patients.** *bioRxiv* 2020 <http://dx.doi.org/10.1101/2020.07.18.210120>.
 32. Carcaterra M, Caruso C: **Alveolar epithelial cell type II as main target of SARS-CoV-2 virus and COVID-19 development via NF-Kb pathway deregulation: a physio-pathological theory.** *Med Hypotheses* 2021, **146**:110412.
 33. Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J *et al.*: **ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia.** *J Virol* 2005, **79**:14614-14621.
 34. Chua RL, Lukassen S, Trump S, Hennig BP, Wendisch D, Pott F *et al.*: **COVID-19 severity correlates with airway epithelium-immune cell interactions identified by single-cell analysis.** *Nat Biotechnol* 2020, **38**:970-979.
 35. Short KR, Kasper J, van der Aa S, Andeweg AC, Zaaraoui-Boutahar F, Goeijenbier M *et al.*: **Influenza virus damages the alveolar barrier by disrupting epithelial cell tight junctions.** *Eur Respir J* 2016, **47**:954-966.
 36. Tang BM, Shojaei M, Teoh S, Meyers A, Ho J, Ball TB *et al.*: **Neutrophils-related host factors associated with severe disease and fatality in patients with influenza infection.** *Nat Commun* 2019, **10**:3422.
 37. Narasaraaju T, Yang E, Samy RP, Ng HH, Poh WP, Liew A-A *et al.*: **Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis.** *Am J Pathol* 2011, **179**:199-210.
 38. Rudd JM, Pulavendran S, Ashar HK, Ritchey JW, Snider TA, Malayer JR *et al.*: **Neutrophils induce a novel chemokine receptors repertoire during influenza pneumonia.** *Front Cell Infect Microbiol* 2019, **9**.
 39. Dorward DA, Russell CD, Um IH, Elshani M, Armstrong SD, Penrice-Randal R *et al.*: **Tissue-specific immunopathology in fatal COVID-19.** *Am J Respir Crit Care Med* 2021, **203**:192-201.
 40. Ghoneim HE, Thomas PG, McCullers JA: **Depletion of alveolar macrophages during influenza infection facilitates bacterial superinfections.** *J Immunol* 2013, **191**:1250-1259.
 41. Schulters GS, Zhang M, Fall N, Husami A, Kissell D, Hanosh A *et al.*: **Whole-exome sequencing reveals mutations in genes linked to hemophagocytic lymphohistiocytosis and macrophage activation syndrome in fatal cases of H1N1 influenza.** *J Infect Dis* 2015, **213**:1180-1188.
 42. Webb BJ, Peltan ID, Jensen P, Hoda D, Hunter B, Silver A *et al.*: **Clinical criteria for COVID-19-associated hyperinflammatory syndrome: a cohort study.** *Lancet Rheumatol* 2020, **2**:e754-e763.
 43. Punnith Abdulsamad S, Bhatia K, Khalid H, Makan A, Crawford E, Ahmad N *et al.*: **Cellular response to influenza infection: lymphopenia and a reduced lymphocyte/monocyte ratio.** *Eur Respir J* 2019, **54**(Suppl. 63):PA2383.
 44. Qian F, Gao G, Song Y, Xu Y, Wang A, Wang S *et al.*: **Specific dynamic variations in the peripheral blood lymphocyte subsets in COVID-19 and severe influenza A patients: a retrospective observational study.** *BMC Infect Dis* 2020, **20**:910.
 45. Janssen NAF, Grondman I, de Nooijer AH, Boahen CK, Koeken V, Matzaraki V *et al.*: **Dysregulated innate and adaptive immune responses discriminate disease severity in COVID-19.** *J Infect Dis* 2021, **223**:1322-1333 <http://dx.doi.org/10.1093/infdis/jiab065>
- This study investigates the immunological profile of COVID-19 patients using a comprehensive proteomics approach.
46. Bertuzzi M, Hayes GE, Icheoku UJ, van Rhijn N, Denning DW, Oshero N *et al.*: **Anti-aspergillus activities of the respiratory epithelium in health and disease.** *J Fungi* 2018, **4**:8.
 47. Zhang Z, Liu R, Noordhoek JA, Kauffman HF: **Interaction of airway epithelial cells (A549) with spores and mycelium of *Aspergillus fumigatus*.** *J Infect* 2005, **51**:375-382.
 48. Gazendam RP, van Hamme JL, Tool AT, Hoogenboezem M, van den Berg JM, Prins JM *et al.*: **Human neutrophils use different mechanisms to kill *Aspergillus fumigatus* conidia and hyphae: evidence from phagocyte defects.** *J Immunol* 2016, **196**:1272-1283.
 49. Ibrahim-Granet O, Philippe B, Boleti H, Boisvieux-Ulrich E, Grenet D, Stern M *et al.*: **Phagocytosis and intracellular fate of *Aspergillus fumigatus* conidia in alveolar macrophages.** *Infect Immun* 2003, **71**:891-903.
 50. Grimm MJ, Vethanayagam RR, Almyroudis NG, Dennis CG, Khan ANH, D'Auria AC *et al.*: **Monocyte- and macrophage-targeted NADPH oxidase mediates antifungal host defense and regulation of acute inflammation in mice.** *J Immunol* 2013, **190**:4175-4184.
 51. Dubourdeau M, Athman R, Balloy V, Huerre M, Chignard M, Philpott DJ *et al.*: ***Aspergillus fumigatus* induces innate immune responses in alveolar macrophages through the MAPK pathway independently of TLR2 and TLR4.** *J Immunol* 2006, **177**:3994-4001.
 52. Mircescu MM, Lipuma L, van Rooijen N, Pamer EG, Hohl TM: **Essential role for neutrophils but not alveolar macrophages at early time points following *Aspergillus fumigatus* infection.** *J Infect Dis* 2009, **200**:647-656.
 53. Vlahos R, Stambas J, Bozinovski S, Broughton BR, Drummond GR, Selemidis S: **Inhibition of Nox2 oxidase activity ameliorates influenza A virus-induced lung inflammation.** *PLoS Pathog* 2011, **7**:e1001271.
 54. Sun K, Metzger DW: **Influenza infection suppresses NADPH oxidase-dependent phagocytic bacterial clearance and enhances susceptibility to secondary methicillin-resistant *Staphylococcus aureus* infection.** *J Immunol* 2014, **192**:3301-3307.
 55. Segal BH, Romani LR: **Invasive aspergillosis in chronic granulomatous disease.** *Med Mycol* 2009, **47** Suppl. 1:S282-90.
 56. van de Veerdonk FL, Dinarello CA: **Deficient autophagy unravels the ROS paradox in chronic granulomatous disease.** *Autophagy* 2014, **10**:1141-1142.

57. Sprenkeler EG, Gresnigt MS, van de Veerdonk FL: **LC3-associated phagocytosis: a crucial mechanism for antifungal host defence against *Aspergillus fumigatus***. *Cell Microbiol* 2016, **18**:1208-1216.
 58. Chamilos G, Akoumianaki T, Kyrmizi I, Brakhage A, Beauvais A, Latge JP: **Melanin targets LC3-associated phagocytosis (LAP): a novel pathogenetic mechanism in fungal disease**. *Autophagy* 2016, **12**:888-889.
 59. Kyrmizi I, Gresnigt MS, Akoumianaki T, Samonis G, Sidiropoulos P, Boupas D *et al.*: **Corticosteroids block autophagy protein recruitment in *Aspergillus fumigatus* phagosomes via targeting dectin-1/Syk kinase signaling**. *J Immunol* 2013, **191**:1287-1299.
 60. Mudd PA, Crawford JC, Turner JS, Souquette A, Reynolds D, Bender D *et al.*: **Distinct inflammatory profiles distinguish COVID-19 from influenza with limited contributions from cytokine storm**. *Sci Adv* 2020, **6**:eabe3024.
- This paper compares the inflammatory characteristics of patients with severe influenza and COVID-19.
61. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N *et al.*: **Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients**. *Science* 2020, **369**:718-724.
 62. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann H-H, Zhang Y *et al.*: **Autoantibodies against type I IFNs in patients with life-threatening COVID-19**. *Science* 2020, **370**:eabd4585.
- This study highlights the presence of auto-antibodies against Type I IFNs in COVID-19 patients.
63. Espinosa V, Dutta O, McElrath C, Du P, Chang Y-J, Cicciarelli B *et al.*: **Type III interferon is a critical regulator of innate antifungal immunity**. *Sci Immunol* 2017, **2**:eaan5357.
 64. Gafa V, Remoli ME, Giacomini E, Severa M, Grillot R, Coccia EM: **Enhancement of anti-*Aspergillus* T helper type 1 response by interferon- β -conditioned dendritic cells**. *Immunology* 2010, **131**:282-288.
 65. Davies BE: **Pharmacokinetics of oseltamivir: an oral antiviral for the treatment and prophylaxis of influenza in diverse populations**. *J Antimicrob Chemother* 2010, **65**(Suppl. 2):ii5-ii10.
 66. Dewi IMW, Cunha C, Gkoutzinopoulou ME, Jaeger M, Gresnigt MS, Duarte-Oliveira C *et al.*: **Neuraminidase and SIGLEC15 modulate the host defense against pulmonary aspergillosis**. *Cell Rep Med* 2021, **2** <http://dx.doi.org/10.1016/j.xcrm.2021.100289> Online ahead of print.
 67. Nyga R, Maizel J, Nseir S, Chouaki T, Milic I, Roger PA *et al.*: **Invasive tracheobronchial aspergillosis in critically ill patients with severe influenza. A clinical trial**. *Am J Respir Crit Care Med* 2020, **202**:708-716.
- This study highlights invasive *Aspergillus* tracheobronchitis in critically ill patients with severe influenza.
68. Stone JH, Frigault MJ, Serling-Boyd NJ, Fernandes AD, Harvey L, Foulkes AS *et al.*: **Efficacy of tocilizumab in patients hospitalized with Covid-19**. *N Engl J Med* 2020, **383**:2333-2344.
 69. Salama C, Han J, Yau L, Reiss WG, Kramer B, Neidhart JD *et al.*: **Tocilizumab in patients hospitalized with Covid-19 pneumonia**. *N Engl J Med* 2020, **384**:20-30.
 70. Witting C, Quaggin-Smith J, Mylvaganam R, Peigh G, Angarone M, Flaherty JD: **Invasive pulmonary aspergillosis after treatment with tocilizumab in a patient with COVID-19 ARDS: a case report**. *Diagn Microbiol Infect Dis* 2021, **99**:115272.
 71. Cauchois R, Koubi M, Delarbre D, Manet C, Carvelli J, Blasco VB *et al.*: **Early IL-1 receptor blockade in severe inflammatory respiratory failure complicating COVID-19**. *Proc Natl Acad Sci U S A* 2020, **117**:18951-18953.
 72. Kooistra EJ, Waalders NJB, Grondman I, Janssen NAF, de Nooijer AH, Netea MG *et al.*: **Anakinra treatment in critically ill COVID-19 patients: a prospective cohort study**. *Crit Care* 2020, **24**:688.
- This paper highlights the beneficial role of anakinra in the treatment of COVID-19 patients.
73. Pasin L, Cavalli G, Navalesi P, Sella N, Landoni G, Yavorovskiy AG *et al.*: **Anakinra for patients with COVID-19: a meta-analysis of non-randomized cohort studies**. *Eur J Intern Med* 2021. S0953-6205(21)00016-9.
 74. Franzetti M, Forastieri A, Borsa N, Pandolfo A, Molteni C, Borghesi L *et al.*: **IL-1 receptor antagonist anakinra in the treatment of COVID-19 acute respiratory distress syndrome: a retrospective, observational study**. *J Immunol* 2021, **206**:1569-1575.
 75. Ni YN, Chen G, Sun J, Liang BM, Liang ZA: **The effect of corticosteroids on mortality of patients with influenza pneumonia: a systematic review and meta-analysis**. *Crit Care* 2019, **23**:99.
 76. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A *et al.*: **Dexamethasone in hospitalized patients with Covid-19**. *New Engl J Med* 2020, **384**:693-704.
- This clinical trial highlights the successful use of dexamethasone in reducing mortality in COVID-19 patients.
77. Boumaza A, Gay L, Mezouar S, Diallo AB, Michel M, Desnues B *et al.*: **Monocytes and macrophages, targets of SARS-CoV-2: the clue for Covid-19 immunoparalysis**. *bioRxiv* 2020 <http://dx.doi.org/10.1101/2020.09.17.300996>.
 78. Delsing CE, Gresnigt MS, Leentjens J, Preijers F, Frager FA, Kox M *et al.*: **Interferon-gamma as adjunctive immunotherapy for invasive fungal infections: a case series**. *BMC Infect Dis* 2014, **14**:166.
 79. Tobin JM, Nickolich KL, Ramanan K, Pilewski MJ, Lamens KD, Alcorn JF, Robinson KM: **Influenza suppresses neutrophil recruitment to the lung and exacerbates secondary invasive pulmonary aspergillosis**. *J Immunol* 2020, **205**:480-488 [jiaa006](https://doi.org/10.1093/infdis/jiaa006).