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Original Article

Isolation of *Aspergillus* in three 2009 H1N1 influenza patients

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Objectives: To describe the association of *Aspergillus* with influenza.

Design/Setting/Sample Three case reports of ICU patients with influenza complicated by the isolation of *Aspergillus* species are described and a review of the literature on the topic was performed.

Conclusions: Severe influenza cases can be complicated by *Aspergillus* infection.

Keywords *Aspergillus*, influenza, pandemic.

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Introduction

The advent of the 2009 H1N1 influenza virus pandemic and the heightened identification of cases that have characterized this pandemic – the 1st in the modern era of sophisticated diagnostics and advanced intensive care – afford the opportunity to witness unusual manifestations of influenza. While certain 'rare' complications may have occurred with some regularity in the past, their occurrence may have went unnoticed because of less aggressive diagnostic methods.

While secondary bacterial pneumonia is a well-known complication of influenza and is deemed responsible for the majority of deaths during the 1918 pandemic,[1] secondary fungal infection – specifically, invasive aspergillosis (IA) – has also been known to play a role in severe influenza cases. Compilations of clinical data on the 2009 H1N1 influenza patients, to date, have mentioned IA as a complicating factor in only two cases.[2–5]

Invasive *Aspergillus* infections are well-known complications of immunocompromised states and are a major burden in solid organ and hematopoietic stem cell transplant populations as well as those afflicted with structural lung disorders.[6] *Aspergillus* is not traditionally thought of as a pathogen capable of causing acute pulmonary symptomatology following a viral respiratory infection; however, cases of IA have been known to complicate influenza since the 1950s.[7] We report three cases that occurred amidst the 2009 H1N1 influenza pandemic at a large tertiary academic medical center and its affiliates.

Case 1

A 52-year-old male with a past medical history significant for gastroesophageal reflux disease and hypertension presented to a community hospital with a 5-day history of fevers, chills, and rigors. He was subsequently diagnosed with pneumonia with respiratory failure necessitating mechanical ventilation, and broad-spectrum antibiotics were initiated and continued through the hospital course (vancomycin, cefepime, and azithromycin). A rapid influenza antigen test was negative at this time. Secondary to inability to maintain oxygenation despite mechanical ventilation, he was transferred to our facility after 1 day. On arrival, influenza was suspected and he was placed on empiric oseltamivir; a subsequent PCR test was positive for influenza A H1N1. On arrival, his absolute lymphocyte count was 600 cells/μl (normal range: 1700–3500 cells/μl). An Immuknow T-cell function assay revealed severe immunosuppression (5 ng ATP/ml, normal range >525 ng

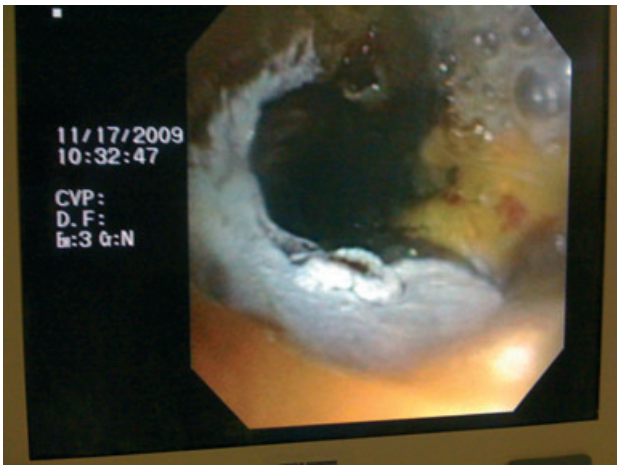

Figure 1. Bronchoscopic image showing adherent plaque to bronchus.

ATP/ml). Owing to refractory hypoxemia, the patient was placed on extracorporeal membrane oxygenation (ECMO) and peramivir was substituted for oseltamivir. The patient's condition did not improve and he was unable to be weaned from ECMO; therefore, a percutaneous bronchoscopically assisted tracheostomy was performed. During the bronchoscopy, adherent white plaques were noted in the airways (Figure 1), which were sampled for microbiologic diagnosis. The patient was placed on fluconazole for suspected fungal infection. Cultures revealed *Candida albicans* as well as *Aspergillus fumigatus*. The patient's antifungal agent was changed to amphotericin B lipid complex after 1 day of fluconazole. A contrasted CT scan of the chest revealed diffuse bilateral pneumonia without evidence of cavitary or nodular lesions (Figure 2). On the same day, the patient developed mental status changes that were confirmed to be secondary to multiple watershed area cerebral infarcts. At that time, the patient's family elected to withdraw care and the patient expired. An autopsy was not obtained. The patient met criteria for possible IA, as the adherent plaque may have been the result of *Candida* infection and the CT findings were not characteristic of IA.

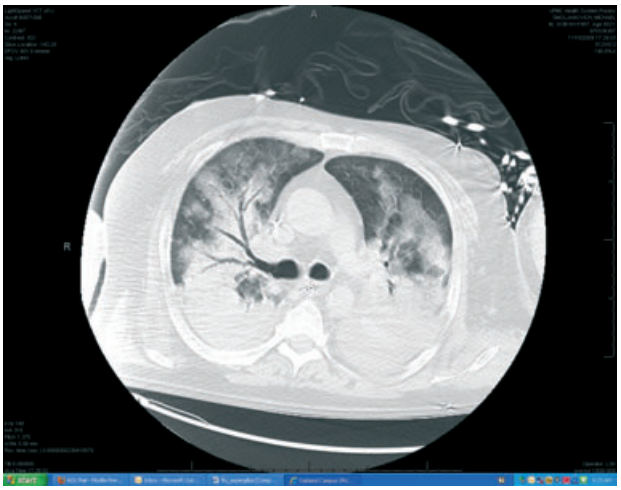

Figure 2. CT of chest revealing diffuse bilateral pneumonia without cavity formation.

Case 2

A 48-year-old male without past medical history presented to a community hospital with a 3-day history of flu-like symptoms. He was subsequently diagnosed with severe pneumonia with bilateral pulmonary infiltrates necessitating mechanical ventilation. A rapid influenza antigen test was negative at this time. Broad-spectrum antibiotics were initiated and continued through the hospital course (ceftriaxone, azithromycin, piperacillin–tazobactam, levofloxacin, and vancomycin). He was transferred to our facility after 1 day. On arrival, influenza was suspected and he was placed on empiric oseltamivir; a subsequent PCR test was positive for influenza A H1N1. His absolute lymphocyte count was 500 cells/μl (normal range: 1700–3500 cells/μl). The patient's condition stabilized over the next several days until he began to have febrile episodes. A bronchoscopy with BAL was performed, and culture was positive for *A. fumigatus*. The serum galactomannan result was 0·1 (normal range <0·5). Voriconazole therapy was commenced, and the patient completed a 28-day course. An Immuknow T-cell assay revealed moderate immune response (274 ng ATP/ml, normal range >525 ng ATP/ml). His T-cell subsets proportions were within normal limits. The patient survived his illness. His case meets criteria for possible IA.

Case 3

A 45-year-old female with a history of mild intermittent asthma and sinus polyps was admitted to the hospital with a several day history of cough, muscle aches, and fever. She had seen her primary physician prior to presentation and was prescribed oseltamivir and azithromycin, but the prescriptions were never filled. Her condition deteriorated, and she presented to the ED where she was found to be febrile, hypoxemic, and in severe respiratory distress. Her WBC count on presentation was 3500 cells/μl (normal range: 4500–10 000 cell/μl) with a lymphocyte count of 385 cells/μl (normal range: 1700–3500 cells/μl). Her T-lymphocytes subsets were within normal limits. She was admitted to the ICU, placed on BiPAP ventilation, and treated with oseltamivir. Broad-spectrum antibiotics were also initiated and continued through her hospital course (ceftriaxone, azithromycin, vancomycin, piperacilin/tazo-

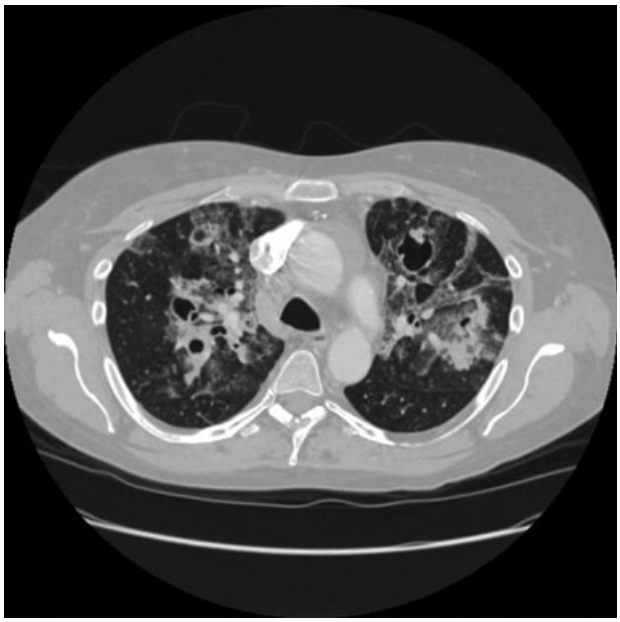

Figure 3. CT of chest revealing cavities, bronchiectatic changes, and extensive consolidation.

bactam, azithromycin, and linezolid). A PCR test confirmed influenza A H1N1. She gradually improved, but after 2 weeks in the hospital developed a new fever. Venous duplex scanning of the legs revealed bilateral lower extremity thrombosis. A CT scan of the chest revealed pulmonary emboli as well as necrotic cavities, bronchiectatic changes, and extensive consolidation (Figure 3). Her CT findings were consistent with IA. The patient underwent a video assisted thorascopic surgery (VATS) procedure as well as bronchoscopy. Bronchoscopy revealed thick mucopurulent secretions, cultures of which grew *A. fumigatus*. The intraoperative findings were of a thickened appearing lung with a firm nodular appearance as well as purulent discharge. The pathologic findings were of an acute and chronic organizing pneumonia with fungal hyphae present, as well as organizing thrombus, proving IA. The patient was placed on a liposomal preparation of amphotericin B for 6 weeks. Her postoperative course was complicated by a bronchopleural fistula requiring a repeat VATS procedure. She survived her illness. Of note, building construction was being undertaken at the facility at which she was treated.

The first published case report

The first published case report of *Aspergillus* in an influenza patient appeared in 1952. Salient features of the case included no known predisposing factor, an influenza-like illness amidst a pandemic, lymphopenia, and a fatal outcome. *Aspergillus* was identified in the postmortem samples of cavitary lung tissue.[7]

Other case reports

Since 1952, several other case reports describing this particular coinfection have appeared. The vast majority of reports describe fatal cases. Major clinical findings reported include:

- There was no evidence of prior immunosuppressed states.
- Many patients were without structural lung disease, although cases occurring in those with chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) have occurred.
- A high proportion of patients had lymphopenia – a known complication of influenza and a risk factor for IA.
- Diagnosis of IA was often made by sputum culture, biopsy findings, or both.
- Influenza was diagnosed predominantly by serology.
- Treatment chiefly consisted of amphotericin B.
- A large proportion of cases are reported in the Japanese literature.

Since 1952, 27 cases of *Aspergillus* associated with influenza have been reported. Salient features include the predominant occurrence with both influenza A; associated lymphopenia; an age range of 14–89 years (median 56 years); the isolation of both *A. fumigatus* and *A. niger*; and eight deaths.[8–23]

Community-acquired aspergillosis

Clancy and Nguyen, in 1999, published a series of 12 cases of acute community-acquired pneumonia in immunocompetent patients caused by *Aspergillus* species. Of the cases reviewed, three were attributed to influenza infection while *all* cases presented with influenza-like illnesses (ILIs). Unfortunately, the results of influenza diagnostic testing were not included in the series.[24] Similarly, the potential role of respiratory viruses other than influenza, given their shared ability to damage respiratory epithelium, was also not reported.

Proposed pathogenesis

The proposed mechanism of predisposition to IA in patients infected with the influenza virus is centered on lymphopenia, a condition that is often generated by influenza virus infection and a possible risk factor for IA – although not as important as phagocyte defects.[6,24] The lymphopenia is primarily driven by viral-induced apoptosis of T lymphocytes.[25–27] In a few of the cases described earlier, diminished numbers of T cells, alterations in the ratio of T-cell subsets, and diminished T-cell function were dem-

onstrated, possibly heightening the risk for IA given that T-cell immunity is important in the control of *Aspergillus* infections.[6,10,12] The dysfunction of surviving T lymphocytes is thought to be mediated by several mechanisms, including infection of T lymphocytes and decreased macrophage/monocyte stimulation of T lymphocytes induced by the infection of macrophages and monocytes.[28,29] Our patients also demonstrated some of these features. Additionally, the alteration in normal mucociliary clearance and destruction of airway epithelium during influenza infection are also postulated to play an equally important role.[24] The role of antibacterial agents in fostering an environment ripe for colonization and subsequent infection with *Aspergillus* should also be considered as a contributing factor**.** Although not utilized in our cases, the use of corticosteroid therapy may diminish the function of neutrophils and macrophages in a manner that is also be conducive to *Aspergillus* infection (e.g. impaired phagocytosis) as well as enhance the growth of the organism.[30]

IA in 2009 H1N1

In the aftermath of the 2009 pandemic and in planning for future pandemics, understanding the contribution and role of co-pathogens (bacterial, viral, and fungal) will be essential to medical surge planning as well as to the need for medical countermeasures that go beyond standard antivirals and extend to agents targeted at other types of microbes. With the large burden of cases, ascertainment of risk factors for specific secondary pathogens and the incremental morbidity incurred may facilitate more accurate prediction of prognosis.

While the great majority of the reported cases of *Aspergillus* isolation in influenza patients were in patients who succumbed to their illness, it is unclear what role *Aspergillus* plays in this process (of note, only one of our cases is a 'proven' IA case). It also remains to be established whether the criteria for IA diagnosis (which were developed for use in severely immunosuppressed patients (e.g. those undergoing chemotherapy for hematological malignancies)[31] are applicable to influenza patients who experience a functionally different and only transient immune deficit. The difficulty in establishing a proven diagnosis of IA in these lower-risk patients is evidenced by two of our cases. *Aspergillus* could be considered a marker for more severe illness secondary to influenza causing structural aberrations in the respiratory tract fostering colonization and invasion with *Aspergillus*. However, given the severity of disease that characterizes IA and the lymphopenia that occurs with influenza, the isolation of *Apergillus* in respiratory samples, a largely forgotten opportunistic co-pathogen, should merit consideration of IA with cavitary pneumonia or pneumonias that are poorly responsive to antibacterial and antiviral therapy.

Conflict of Interest

No conflict of interests.

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