

# Invasive Aspergillosis Associated With Severe Influenza Infections

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**Background.** Invasive aspergillosis may occur in the setting of severe influenza infections due to viral-induced respiratory epithelium disruption and impaired immune effects, but data are limited.

**Methods.** A retrospective study was conducted among severe influenza cases requiring medical intensive care unit (ICU) admission at an academic center during the 2015–2016 season. Data collected included respiratory cultures, medical conditions and immunosuppressants, laboratory and radiographic data, and outcomes. A systematic literature review of published cases in the English language of aspergillosis complicating influenza was conducted.

**Results.** Six (75%) of 8 ICU influenza cases had *Aspergillus* isolated; 5 were classified as invasive disease. No ICU patient testing negative for influenza infection developed aspergillosis during the study period. Among cases with invasive aspergillosis, influenza infection was type A (H1N1) (n = 2) and influenza B (n = 3). Published and current cases yielded n = 57 (European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group criteria: 37% proven, 25% probable, and 39% possible cases). An increasing number of cases were reported since 2010. Sixty-five percent of cases lacked classic underlying conditions at admission for aspergillosis, 86% had lymphopenia, and 46% died.

**Conclusions.** Aspergillosis may occur in the setting of severe influenza infections even among immunocompetent hosts. Risks may include influenza A (H1N1) or B infections and viral-induced lymphopenia, although further studies are needed. Prompt diagnosis and antifungal therapy are recommended given high mortality rates.

**Keywords.** aspergillosis; *Aspergillus*; influenza; review; superinfection.

Bacterial superinfections are well described complications of influenza infections and often contribute to excess morbidity and mortality [1]. Bacterial pathogens often include *Streptococcus pneumoniae* and *Staphylococcus aureus*, including methicillin-resistant strains (methicillin-resistant *S aureus*) [2]. A potential complication of influenza is superinfection with *Aspergillus* species.

Invasive aspergillosis typically occurs in the setting of severely immunosuppressed hosts, including those with T-cell abnormalities such as those with hematologic malignancies, neutropenia, and transplant recipients. As such, the isolation of *Aspergillus* sp in the immunocompetent host without these conditions may be initially considered as a colonizer or non-pathogen. However, recent cases have suggested that *Aspergillus* may rapidly lead to invasive disease in the setting of severe

influenza infection including among those without classic risk factors [3, 4]. The pathogenesis of invasive aspergillosis in this setting may include viral disruption of the respiratory epithelium and impaired local immunity as well as viral-induced systemic Th1/Th2 changes and lymphopenia [5–11]. In addition, the use of concurrent antibiotics that alter the upper respiratory tract flora and steroids via immunosuppressive affects may be risk factors [12–14].

Although sporadic case reports of invasive aspergillosis in the setting of an influenza infection have been published [3, 4, 14], robust data are sparse, especially among previously immunocompetent hosts. This report describes the occurrence of invasive *Aspergillus* complicating severe influenza infections among patients admitted to the intensive care unit (ICU) at a large academic hospital during the 2015–2016 influenza season. In addition, a comprehensive review of the English literature is provided to describe the risk factors and outcomes of this superinfection.

## METHODS

A case-control study was conducted among patients tested for influenza infection who were admitted to the medical ICU at a large academic hospital (400-bed) during the 2015–2016 influenza season. All ICU patients with influenza testing performed via nasopharyngeal swab polymerase chain reaction (PCR) or

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respiratory viral culture were examined. A case was defined as a patient with confirmed influenza infection who subsequently had a positive respiratory culture for *Aspergillus*, and a control was defined as a patient with confirmed influenza infection without evidence of *Aspergillus* on respiratory culture during the hospitalization. All cases and controls had confirmed influenza and were admitted to the ICU with respiratory failure.

Data were retrospectively collected from the electronic medical records among all cases and controls including demographics, underlying medical conditions, and use of immunosuppressive agents before admission. All respiratory culture data during the hospitalization were evaluated (sputum, endotracheal, and bronchial specimens), and bacterial and fungal pathogens were recorded. Laboratory data collected included influenza type and absolute lymphocyte count. Data on the use of steroids during the hospitalization (occurring before the diagnosis the *Aspergillus* in superinfected cases) and influenza treatment were recorded. Chest computed tomography (CT) and radiograph imaging were reviewed. Days of hospitalization, days of required ventilator support, mortality before discharge, time to death, and cause of death (when applicable) were collected. For those with aspergillosis, data on the number of days between the diagnosis of influenza and aspergillosis, *Aspergillus* species, bronchoscopy findings (if performed), supporting laboratory findings for the diagnosis of aspergillosis (galactomannan and  $\beta$ -glucan levels), and antifungal therapy were recorded. Autopsies were not performed on the cases or controls, so these data were not available.

Aspergillosis was defined using the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) consensus guidelines [15]. Because these guidelines were largely created for immunosuppressed hosts (ie, hematologic malignancies and hematopoietic stem cell transplant recipients), and influenza-related aspergillosis may occur in previously normal hosts, criteria for immunosuppression was not required in the present study. In addition, because *Aspergillus* infections in this setting may not initially present with cavitary disease (a criteria for “probable disease”), all cases were captured including those with noncavitary lung disease that met the “possible” definition. All cases met mycological criteria to be included [15].

In addition to the case-control study, a systematic review (PubMed January 1963–March 2016) of the English published literature using the search terms “influenza” and “aspergillus” or “aspergillosis” was conducted. In addition, references of identified papers were used as sources to identify additional cases. Only cases of confirmed influenza as demonstrated by a positive influenza viral culture, PCR, or serology were included. Cases involving nonspecific viral infections or without confirmatory influenza testing, reports with insufficient patient information, and instances in which the *Aspergillus* diagnosis

preceded influenza infection were excluded. The same data, if available, were abstracted from the published cases as for the present case-control study, as described above. Cases in the literature were combined with the current cases to provide a detailed summary of patients with invasive *Aspergillus* in the setting of influenza.

Statistical analyses included descriptive statistics including numbers (percentages) and medians (ranges) for categorical and continuous variables, respectively. Comparisons were made using independent *t* tests and  $\chi^2$  testing (Fisher’s exact tests were used if the cell size was <5), and statistical significance was defined as a  $P < .05$ . Data analyses were performed using SPSS (IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY: IBM Corp., 2013).

## RESULTS

### Case-Control Study

In the case-control study, 48 medical ICU patients underwent influenza testing (nasopharyngeal PCR and/or respiratory viral culture), and 8 were diagnosed with influenza infection (Table 1). All influenza-positive patients had ventilator-dependent respiratory failure. Of the 8 patients with severe influenza infection, 6 (75%) had *Aspergillus* spp (4 *Aspergillus fumigatus*, 1 *A fumigatus* and *Aspergillus versicolor*, and 1 *Aspergillus niger*) isolated. Of the 40 patients negative for influenza admitted to the same ICU, none had *Aspergillus* spp on any of the respiratory cultures (11 had a bacterial pathogen, and 2 had non-*Aspergillus* fungal pathogens likely acquired preadmission [*Cryptococcus* and *Coccidioides* spp]).

Because *A niger* was of unknown pathogenicity and the patient was made comfort care before further evaluation was performed, this case was excluded. Of the 5 patients with influenza infection who had *Aspergillus* sp superinfection, the median age was 59 years (range, 47–86) and all were male (Table 1). None had an immunosuppressive underlying medical condition, and none were receiving immunosuppressant agents before admission. All patients had at least 1 underlying medical condition, including 2 with underlying lung disease (ie, asthma), as shown in Table 1.

The influenza type was A (H1N1) ( $n = 2$ ) and influenza B ( $n = 3$ ), and all patients received anti-influenza medications. The timing between influenza diagnosis and the first positive culture for *Aspergillus* was a median 3 days (range, 0–8). All except 1 of the superinfected patients had lymphopenia (median, 520/ $\mu$ L). One patient received steroids while in the ICU to treat severe pneumonia and asthma exacerbation; however, the remainder of cases did not receive any immunosuppressant medications before the diagnosis of aspergillosis (Table 1).

All aspergillosis cases had diffuse pulmonary consolidative infiltrates, and all had at least 1 positive respiratory culture for *Aspergillus*. In addition, case 1 had fungal tracheobronchitis seen on bronchoscopy examination at 3 separate time points

**Table 1. Case-Control Study of Influenza With *Aspergillus* Superinfection Among ICU Patients, 2015–2016**

Case/Control	Age	Sex	Underlying Medical	Immunosuppressive Medications on Admission	Influenza Type	Influenza Treatment	Time to <i>Aspergillus</i> From Influenza	Steroids Before <i>Aspergillus</i>	Lymphocyte count/ $\mu$ L	<i>Aspergillus</i> Species	CT Findings	Antifungal Treatment	Time to Death	Cause of Death
<b><i>Aspergillus</i> Cases</b>														
Case 1	47	M	RAD	None	A (H1N1)	Oseltamivir 150 mg bid x 7 d, then Peramivir for 5 d	6 d	Yes, prednisone equivalent of 75 mg/d	397	<i>Aspergillus fumigatus</i>	Dense consolidations in all lobes	Voriconazole and then micafungin	NA	NA
Case 2	86	M	CKD, CHD	None	B	Oseltamivir 150 mg bid x 10 d	8 d	No	3470	<i>A fumigatus</i>	Bilateral consolidations on CXR; CT not done	Voriconazole	22	Respiratory failure, sepsis
Case 3	57	M	DM, HTN	None	B	Oseltamivir 150 mg bid x 5 d	3 d	No	114	<i>A fumigatus</i> and <i>Aspergillus versicolor</i>	Patchy nodular infiltrates in all lobes	Voriconazole	29	ARDS, respiratory failure, MOF
Case 4	59	M	Alcoholic liver disease	None	B	Oseltamivir 150 mg bid x 8 d	0 d	No	780	<i>A fumigatus</i>	Bilateral infiltrates in all lobes; 1.4 cm paratracheal node	Voriconazole, and then micafungin	NA	NA
Case 5	62	M	RAD, acute perforated duodenum	None	A (H1N1)	Peramivir iv x 3 d, then Oseltamivir 150 mg bid x 7 d	2 d	No	520	<i>A fumigatus</i>	Bilateral upper and lower lobe opacities	Voriconazole	13	Respiratory failure, sepsis
<b>Single positive culture without clear invasive disease</b>	77	M	NHL, COPD, HCV, CHD	Chemotherapy	A (H1N1)	Oseltamivir 150 mg bid x 10 d	14 d	No	210	<i>Aspergillus niger</i>	Right upper lobe infiltrate on CXR; CT not done	None; comfort care	NA; patient was discharged on home hospice	NA
<b>No evidence of <i>Aspergillus</i></b>														
Control 1	60	F	Obesity, duodenal perforation CHD, HTN	None	A (H1N1)	Peramivir iv for 4 d	NA	No	1240	NA	Bilateral consolidations	NA	13	Respiratory failure, sepsis
Control 2	83	M	DM, HTN, CVA	None	A (H3)	Oseltamivir 150 mg bid x 2 d	NA	Yes, hydrocortisone 50 mg/d	950	NA	Ground-glass infiltrates in upper lobes; bilateral lower lobe consolidations	NA	3	Respiratory failure, sepsis, cardiac arrest

Abbreviations: ARDS, acute respiratory distress syndrome; CHD, chronic heart disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CT, computed tomography; CVA, cerebrovascular disease; CXR, chest radiograph; DM, diabetes mellitus; F, female; HCV, hepatitis C virus; HTN, hypertension; ICU, intensive care unit; iv, intravenous; MOF, multiorgan failure; NA, not applicable; NHL, non-Hodgkin's lymphoma; RAD, reactive airway disease.

(Figure 1) as well as branching hyphae consistent with aspergillosis on pathology. Galactomannan and/or  $\beta$ -glucan levels were evaluated in 4 of the patients, and 3 (75%) had positive results. Case 1 also had a positive bronchoalveolar lavage (BAL) galactomannan. Overall, case 1 met the EORTC/MSG criteria for probable aspergillosis, and cases 2–5 met the criteria for possible aspergillosis.

Despite prompt administration of antifungal therapy in each case, mortality occurred in 3 of 5 (60%) patients with concurrent influenza and *Aspergillus*. The 2 survivors had significant postinfection morbidities: case 1 had persistent ventilator-dependent respiratory failure at time of transfer (>60 days after admission) and also suffered from critical care polyneuropathy with resultant quadriplegia and renal failure requiring ongoing hemodialysis. Case 4 had ventilator-dependent respiratory failure >30 days after admission. Both cases required tracheostomy and G-tubes, and both remained ventilator-dependent at time of transfer to skilled nursing facilities. A description of influenza cases with and without *Aspergillus* is shown in Table 1; formal analyses were not possible because there were only 2 patients in the latter group.

#### Review of the Literature

The systematic review of the literature ( $n = 52$ ) [3, 4, 14, 16–38] and the present cases ( $n = 5$ ) totaled 57 cases. A summary of the cases is shown in Table 2, and the full data are shown in Supplementary Table 1. There was an increasing number of cases published in the literature over time: the first cases were described in 1979, followed by 3 cases in the 1980s, 2 cases in the 1990s, 2 cases in the 2000s, and 48 cases from 2010 to the first quarter of 2016, demonstrating an increasing trend of aspergillosis superinfection ( $P < .001$ ).



**Figure 1.** Fungal tracheobronchitis consistent with invasive aspergillosis.

The median age was 53 years (range, 23–86) and 67% were males. Overall, 46 (81%) had at least 1 underlying medical condition reported. Only approximately one third of patients had an underlying condition classically associated with invasive aspergillosis (eg, hematologic malignancy, transplantation, neutropenia). The most common conditions were cancer ( $n = 12$ ; most commonly leukemia [ $n = 8$ ]), neutropenia ( $n = 9$ ), and transplant receipt ( $n = 8$ ). Other underlying medical conditions included diabetes ( $n = 9$ ), chronic obstructive pulmonary disease or asthma ( $n = 7$ ), hypertension ( $n = 6$ ), alcoholism ( $n = 4$ ), liver disease ( $n = 3$ ), obesity ( $n = 3$ ), tobacco use ( $n = 3$ ), cardiac disease ( $n = 2$ ), and single cases with human immunodeficiency virus/acquired immune deficiency syndrome, rheumatoid arthritis, myasthenia gravis, chronic kidney disease, and thrombotic thrombocytopenic purpura; some cases had multiple underlying conditions.

At admission, 32% were receiving immunosuppressant agents; 9 patients were on chemotherapy, whereas others were receiving other types of immunosuppressants including steroids ( $n = 10$ ), tacrolimus or related agents ( $n = 4$ ), cyclosporine ( $n = 2$ ), and mycophenolate ( $n = 1$ ); some were on multiple immunosuppressive medications. All cases receiving immunosuppressants at the time of admission also had an underlying classic immunosuppressive condition (eg, cancer, transplant recipient), except in 2 cases (Table 2). Eighteen (32%) cases received steroids between the time of influenza and *Aspergillus* diagnoses, with 7 receiving steroids prior to admission and 11 receiving steroids exclusively during the hospitalization. Accounting for any of the aforementioned immunosuppressive conditions (ie, underlying immunocompromising condition, immunosuppressant use at admission, and/or steroid use after admission), ~50% of cases had none of these conditions reported. Twenty-two patients had lymphocyte counts reported, and 19 (86%) had lymphopenia as defined as  $<1000/\mu\text{L}$  (median,  $500/\mu\text{L}$ ).

Fifty-three of the cases in the literature occurred in the setting of influenza A and 4 with influenza B. Of the influenza A strains, 40 were H1N1 and 3 were H3 strains (the type was not provided in 10 cases). Since 2010, the majority of cases were due to the A (H1N1) strain, with only 3 due to type B (all reported in the current series), and 2 cases were due to influenza A (H3). Anti-influenza therapy was administered in 30 (53%) cases with all patients receiving oseltamivir (doses of 75–150 mg po twice daily); 3 patients additionally received peramivir, and 1 patient received zanamivir. The total duration of anti-influenza therapy was reported for 10 cases with a median duration of 12 days (range, 5–21 days). For the other 27 cases, anti-influenza therapy was not reported or not administered.

The time between influenza and *Aspergillus* diagnoses was reported in 36 cases and was a median of 6 days (range, 0–32). All cases had pulmonary involvement with 9 cases also with tracheobronchitis and 7 with systemic infection (eg, brain,

**Table 2. Summary of 57 Cases of Invasive *Aspergillus* During Influenza Infection: Current Cases and Literature Review**

Characteristic	Number (%) <sup>a</sup> or Median (Range)
<b>Demographics</b>	
Age, years	53 (23–86)
Sex, male	38 (67%)
<b>Underlying medical conditions at admission</b>	
“Classic underlying disease” <sup>b</sup>	18 (32%)
Underlying condition, yes	46 (81%)
<b>Types of conditions<sup>c</sup></b>	
Cancer	12 (21%)
Neutropenia	9 (16%)
Diabetes	9 (16%)
Transplantation	8 (14%)
Underlying lung disease	7 (12%)
Immunosuppressant use at admission	18 (32%)
Immunosuppressed host (based on condition and/or medication use) at admission	20 (35%)
<b>Influenza-related data at admission</b>	
<b>Type</b>	
A	53 (93%)
B	4 (7%)
Receipt of anti-influenza treatment	30 (53%)
<b>Hospitalization data</b>	
Lymphopenia (<1000 cells/ $\mu$ L)	19/23 (18%)
Steroid use, yes	18 (32%)
<b><i>Aspergillus</i> data</b>	
Days from influenza to <i>Aspergillus</i> diagnosis	6 (0–32) <sup>a</sup>
<b>Sites of Aspergillosis<sup>c</sup></b>	
Lung	57 (100%)
Tracheobronchitis	9 (16%)
Systemic	6 (11%)
<b>Species</b>	
<i>Aspergillus fumigatus</i>	40 (70%)
<i>A fumigatus</i> and <i>Aspergillus versicolor</i>	1 (2%)
<i>A fumigatus</i> and <i>Aspergillus nidulans</i>	1 (2%)
<i>Aspergillus niger</i>	1 (2%)
<i>Aspergillus terreus</i>	1 (2%)
Not reported	13 (23%)
<b>Type of diagnostic specimen<sup>c</sup></b>	
Bronchoscopy culture	27 (47%)
Sputum culture	22 (39%)
Pathology	21 (37%)
Galactomannan	28 (49%)
<i>Aspergillus</i> PCR	4 (7%)
Bronchoscopy lesion	1 (2%)
<b>EORTC/MSG criteria</b>	
Proven	21 (37%)
Probable	14 (25%)
Possible	22 (39%)
Antifungal therapy, yes	53 (93%)
<b>Outcomes</b>	
<b>Mechanical ventilation</b>	
Yes	38 (67%)
No	11 (19%)
Not reported	8 (14%)

Table 2 continued.

Characteristic	Number (%) <sup>a</sup> or Median (Range)
ECMO	12 (21%)
Mortality	26 (46%)

Abbreviations: ECMO, extracorporeal membrane oxygenation; EORTC/MSG, European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group; PCR, polymerase chain reaction; TTP, thrombotic thrombocytopenic purpura.

<sup>a</sup> Denominator is 57 unless otherwise noted; the denominator for time to *Aspergillus* diagnosis was  $n = 36$ .

<sup>b</sup> Classic immunosuppressive condition included leukemia, neutropenia, and/or transplant recipient. All patients with an underlying condition were also on an immunosuppressant medication at admission, except for 2 patients. All patients receiving an immunosuppressant were also diagnosed with a classic underlying medical condition except in 2 cases: myasthenia gravis ( $n = 1$ ) and TTP ( $n = 1$ ) who were receiving steroids.

<sup>c</sup> More than 1 factor may be present, hence the percentage may exceed 100%.

myocardium). The *Aspergillus* species was reported in 44 (77%) cases. *Aspergillus fumigatus* was cultured in the majority of cases ( $n = 42$ ), with 2 also having an additional species isolated (*A versicolor* and *Aspergillus nidulans*). There was 1 case each with solely *A niger* and *Aspergillus terreus*. In the other 13 cases of aspergillosis, the species was not reported (5 of the diagnoses were based on serologic testing [eg, galactomannan], 2 by pathology, 1 by PCR and pathology results, 1 had hyphae on sputum sample, and 4 had no specific information reported). In addition to the isolation of *Aspergillus*, 12 patients had concurrent bacterial respiratory pathogens: 4 *S aureus*, 3 *S pneumoniae*, 2 *Haemophilus influenzae*, 2 *Pseudomonas* sp, 1 *Klebsiella pneumoniae*, and 1 *Acinetobacter* sp; 1 patient had 2 bacterial pathogens isolated.

The diagnosis of aspergillosis was made using a variety of culture, pathology, and blood test results: 14 had positive bronchoscopy culture; 12 with positive bronchoscopy culture plus pathology findings; 13 by sputum culture; 6 by sputum culture and pathology findings; 5 by galactomannan alone; and 2 with sputum plus PCR data. There were single cases of sputum plus brain pathology; bronchoscopy and brain pathology; presence of a characteristic bronchoscopy lesion; pathology plus PCR; and antigen plus PCR data. A serum galactomannan test was evaluated in 36 patients and reported as positive in 28 (78%). A galactomannan of the BAL or other respiratory fluid was reported in 17 cases, and 15 (88%) were positive. Finally,  $\beta$ -glucan was reported in only 5 cases with 4 being positive and 1 indeterminate.

Based on the EORTC/MSG criteria, 21 (37%) cases were proven aspergillosis, 14 (25%) were probable cases, and 22 (39%) were possible cases. Antifungal treatment was initiated in all but 4 cases; 3 of those without therapy died. The specific medication used was noted in 49 cases and varied over the study period. Combinations or sequential antifungal therapy was given in 14 cases, and monotherapy was given in the remaining



cases. The most common antifungal agents used were voriconazole (n = 39), amphotericin B or liposomal amphotericin (n = 16), or an echinocandin (n = 8). Other types of azoles were used in 3 cases (itraconazole = 2, econazole = 1). Two cases used inhaled/nebulized amphotericin, 1 used flucytosine, and 1 used adjunctive granulocyte-macrophage colony-stimulating factor and  $\gamma$ -interferon.

Thirty-eight patients required mechanical ventilation (range, 2 days to >2 years), 11 did not require intubation, and in 8 cases data were not reported. Extracorporeal membrane oxygenation was used in 12 (21%) cases. The total length of hospitalization was reported in 38 cases, and total length of hospitalization for 21 (55%) cases was >30 days. Overall, 26 (46%) cases died. The time to death was reported in 17 cases with a median time of 21 days (range, 7–70). The cause of death was reported for 16 deaths with respiratory failure being the most common cause (n = 8) along with multiorgan failure (n = 6), septic shock (n = 4), central nervous involvement/disease (n = 3), and cardiac arrest (n = 1); some cases had multiple contributing causes. Factors associated with mortality included proven versus possible EORTC/MSG criteria (odds ratio = 5.23,  $P = .03$ ), although this may be partly due to autopsy pathology findings are a criterion for proven disease. No other factors were significantly associated with mortality, including underlying condition, year of diagnosis, steroid use, type or treatment of influenza, lymphopenia, classic versus non-classic CT findings, or fungal treatment.

## DISCUSSION

Aspergillosis may occur in the setting of severe influenza infections with important clinical implications. The current report describes 5 cases in a single ICU during the recent 2015–2016 influenza season as well as an additional 52 cases in the English literature. Since 2010, there have been a rapidly increasing number of cases described in the literature associated with influenza (A [H1N1] and B) and subsequent invasive *Aspergillus* superinfection. Despite that invasive aspergillosis is classically associated with immunosuppressive conditions (eg, leukemia and transplantation), the rising number of recent cases, including among immunocompetent hosts, suggests that influenza may represent a novel host risk factor for this invasive fungal infection.

The reason for the dramatic increase in the number of recently published cases of invasive aspergillosis in the setting of influenza is unclear. One possible hypothesis is the evolution of more virulent influenza strains, as exemplified by the pandemic H1N1 strain, which may cause more severe and diffuse damage to the respiratory mucosa [39, 40] allowing for fungal invasion. A small study among leukemia patients with neutropenia found an increased incidence of invasive aspergillosis after the introduction of H1N1 [22]. Although most cases have been linked to A (H1N1), this series demonstrates that other types, including B, can cause severe influenza disease and superinfection with *Aspergillus*. Another possibility for the rising trends may be

the increasing use of steroids to treat severe pneumonia [13], which may cause immunosuppressive effects linked to invasive fungal infection. Finally, it is possible that there is a greater reporting of cases over time.

The pathogenesis of invasive aspergillosis in the setting of influenza infection may be due to both local and systemic effects of the virus. Local damage to the tracheobronchial mucosa and disruption of normal ciliary clearance have been previously implicated in bacterial superinfections (eg, *S aureus*, *S pneumoniae*) [41] and may also allow colonizing fungi to cause invasive disease. Influenza may also impair local phagocytosis by alveolar macrophages as well as reduce natural killer cell functionality and other immune responses via cytokine imbalances [5–9, 42]. Systemically, influenza affects the Th1/Th2 response and causes lymphopenia, both known risk factors for aspergillosis [9–11, 43]. Although none of the cases in the literature measured cytokines (eg, interleukin-10) or specific Th1/Th2 immune responses, several cases documented lymphopenia at the time of *Aspergillus* diagnosis.

The reasons that some, but not all, patients with severe influenza infection develop aspergillosis remains unknown. The current case-control study was unable to compare these groups due to the small numbers of controls, and the literature has largely reported only patients with *Aspergillus* infection. It is possible that exposure to *Aspergillus* at a critical time of both reduced mucosal and systemic immune defenses subsequently leads to invasive disease. The median time from influenza to *Aspergillus* diagnosis was 6 days. However, the time range was broad (0–32 days) and may be influenced by delays in the diagnosis of both infections. Although some reports have implicated steroid use with occurrence of fungal superinfection [12, 16], including a small series [14], we could not verify this as a risk factor because only approximately one third of cases in the literature reported steroid use preceding *Aspergillus* diagnosis; regardless, cautious use of steroids should be considered until further data are available, and studies to date have not shown that steroids in the setting of influenza pneumonia is associated with better outcomes [12, 44]. In addition, many patients with influenza are initially treated with broad-spectrum antibiotics, which may lead to the loss of normal respiratory flora and provide a potential niche for fungal pathogens. Regarding specific environmental exposures to fungi, none were noted in our study or in prior studies [14]. Overall, further research comparing influenza cases with and without aspergillosis from the same catchment area are needed to understand the precise risk factors for this superinfection. Data on immune function, colonizing flora, and potential risk factors (eg, concurrent medications, including immunosuppressants, and environmental exposures) are needed. Until further data are obtained, reducing potential risk factors for invasive fungal infections is advocated.

The isolation of *Aspergillus* among ventilated patients without classic risk factors for invasive fungal disease may initially

be considered a contaminant or colonizer. This review suggests that invasive aspergillosis may occur in patients with few or no underlying medical conditions. Patients with *Aspergillus* spp isolated in the setting of severe influenza infection should be promptly evaluated for invasive disease. The EORTC/MSG criteria [15] have been used for diagnosing invasive aspergillus among immunocompromised hosts, including those undergoing chemotherapy and transplant recipients; however, the validity of these criteria among other patient groups without “classic” risk factors remains unknown. Among the current review of the literature, most patients with aspergillosis associated with influenza did not have classic risk factors or radiographic findings (eg, cavitary lesions, halo, or crescent signs, which are typically more common in neutropenic and/or transplant patients) [45], rather most commonly had nonspecific multifocal infiltrates or opacities. The diagnostic work-up was diverse among reported cases in the literature; however, one approach is to repeat respiratory cultures (via bronchoscopy, if possible), obtain chest CT imaging, and consider checking serum fungal markers such as galactomannan and  $\beta$ -glucan levels. Although further data on fungal serologic markers in this setting are needed, 80%–90% of cases in the literature that underwent testing had positive results.

The mortality rate among superinfected patients was approximately 50% and did not appear to change over time. This compares with a mortality rate of ~10%–20% among hospitalized patients with influenza alone [43, 46]. Among survivors, morbidity was frequently reported, with some cases having prolonged respiratory failure requiring tracheostomy and ventilator support, including the 2 survivors in the current series. Overall, invasive aspergillosis in the setting of influenza is associated with a high risk of adverse outcomes. Antifungal therapy was administered to most cases (all except 4), and most commonly utilized voriconazole. Concurrent implementation of a diagnostic work-up and antifungal therapy appears warranted given the high morbidity and mortality rates among superinfected patients.

Strengths of this report include the comprehensive review of the English literature (January 1963–March 2016) because most data to date has focused on individual case reports or case series. In addition, data on 5 additional cases were added to the existing literature. Finally, data on a wide range of factors were collected from each published report to provide a robust description of this superinfection. Limitations include the small size of the case-control study from a single academic institution and that many of the published cases did not include specific clinical or laboratory factors of interest. Future work should include (1) larger case-control studies to determine the specific risk factors for invasive aspergillosis in the setting of influenza infection and (2) comprehensive studies to further examine diagnostic criteria and treatment approaches.

## CONCLUSIONS

In summary, *Aspergillus* is a potential complication of severe influenza infections, even among immunocompetent hosts. Progressive pulmonary infection after the initial diagnosis of influenza should raise suspicion of superinfection including with *Aspergillus*. Risks may include infection with influenza A (H1N1) or B and viral-induced lymphopenia; however, further data on the exact pathogenesis are needed. Reducing potential risk factors for invasive aspergillus among severe influenza cases should be considered, including potentially limiting the use of unnecessary antibiotics and steroids. Prompt diagnosis (using respiratory cultures, CT imaging, as well as potentially preempive galactomannan and  $\beta$ -glucan levels) and early antifungal therapy are recommended given the high mortality and morbidity associated with this superinfection.

## Supplementary Data

Supplementary material is available online at *Open Forum Infectious Diseases* online (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

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