Influenza-associated Aspergillosis in Critically Ill Patients

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Published in:
American Journal of Respiratory and Critical Care Medicine

DOI:
10.1164/rccm.201612-2540LE

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To the Editor:

Invasive aspergillosis is a well-known complication in immunocompromised patients but may also develop in patients with influenza pneumonia. The largest reported series included nine cases of invasive aspergillosis among 40 critically ill patients with influenza A H1N1 infection (23%) over a period of 3 years in two centers (1). Influenza-associated aspergillosis (IAA) may develop in immunocompromised patients (1, 2), but it has also been reported in apparently immunocompetent patients and was associated with the use of corticosteroids (1, 3, 4). Review of all 68 cases with IAA reported during the last 60 years indicated an overall mortality rate of 47% (5).

We conducted a multicenter retrospective observational study involving all eight academic tertiary care intensive care units (ICUs) in the Netherlands from December 2015 to April 2016, aimed at describing the diagnosis, treatment, and outcome of IAA in adults. All data were processed anonymously, and the ethics committee waived informed consent.

Influenza cases were identified both by reviewing all patients with a positive influenza polymerase chain reaction in the registry of the local microbiology department and matching these with ICU admission, and via using national ICU registrations of viral pneumonia and oseltamivir treatment to identify patients diagnosed with influenza or treated for influenza at a referring hospital. The IAA case definition included confirmed influenza diagnosis based on a positive result from a reverse transcriptase polymerase chain reaction test for influenza A and B from nasopharyngeal swab, sputum, or bronchoalveolar lavage (BAL) fluid. Furthermore, new infiltrates on the chest X-ray or computed tomography scan had to be present, as well as clinical symptoms including refractory fever or worsening of respiratory insufficiency despite more than 3 days of antibiotic therapy, dyspnea, hemoptysis, and/or pleural friction rub. Mycological evidence included histopathology or direct microscopic evidence of dichotomous branching hyphae with positive culture for *Aspergillus* from tissue. In addition, a galactomannan optical index in BAL of more than 1 or in serum of more than 0.5 within 3 weeks of influenza diagnosis was considered sufficient evidence for the diagnosis of IAA (4). After 3 weeks, other factors, such as corticosteroid use, may contribute substantially to the risk of developing invasive aspergillosis (4). Patients were classified if host, clinical, and mycological factors were present. A web-based case record form was used to collect microbiological and clinical data. For each center, the number of influenza cases admitted to the ICU within the 4-month period was collected. Data were analyzed with SAS 9.2 (SAS Institute Inc., Cary, NC).

Between December 1, 2015, and March 31, 2016, 144 influenza cases were admitted to the ICU of the eight centers, of which 23 (16%) matched our IAA case definition (Table 1). Influenza A was found in 21 patients (91%), and all patients received oseltamivir or zanamivir therapy. Our patient cohort included 14 males and 9 females, and the median age was 62 years (range, 34–80 years; Table 1). A preexisting underlying condition was present in 16 patients (70%), which could be classified as high risk (2 patients), intermediate risk (2 patients), low risk (6 patients), and no risk for invasive fungal disease (4 patients) (6). None were neutropenic. In seven patients (30%), no underlying disease was present at the time of acquiring influenza (Table 1).

Galactomannan was detected in BAL fluid of 17 of 18 patients (94%), whereas BAL culture was positive in 14 patients (78%; Table 1). Serum galactomannan was determined in 14 patients, of whom 10 were positive (71%). In 14 patients, *in vitro* susceptibility testing was performed, and azole resistance was found in 4 (29%), including 3 patients who harbored both azole-susceptible and azole-resistant isolates (7). Fourteen (61%) of 23 patients with IAA died, all during ICU admission. Five (71%) of the 7 previously healthy patients died compared with 9 (56%) of 16 patients with preexisting underlying conditions. In three patients (Table 1), autopsy showed evidence of necrosis and erosion of the epithelium of the trachea and bronchi, with invasive hyphal growth. The median time to initiation of antifungal therapy from the day of the influenza diagnosis was 9 days compared with 2 days in nonsurvivors and survivors, respectively (Mann-Whitney $P = 0.06$; 95% confidence interval for the difference, -0.2 to 13; Figure 1).

We observed IAA in critically ill patients with influenza and identified 11 patients who were previously healthy (seven) or had no known risk for invasive aspergillosis (four). The mortality rate of IAA was high; it was higher than the previously reported 47% mortality rate (5). The mortality rate among patients without risk factors was not lower compared with those with low, intermediate, or high risk for invasive fungal disease. Delayed diagnosis of IAA in the ICU and subsequent delayed antifungal therapy might have contributed to this high mortality. The clinical presentation of IAA differed substantially from presentations seen in patients with classic risk factors. The observation of two patients with *Aspergillus* tracheobronchitis underscores the need to further characterize clinical and radiological features of IAA (8). Furthermore, a high triazole resistance frequency was observed, which appears to be higher in the Netherlands than in other countries and further compromises successful patient management (9).

It is important to understand the pathogenesis of IAA from the perspective of virus, fungus, and host. Strikingly, in line with our observation, almost all cases to date have been associated with the pandemic influenza A H1N1 infection. Although it has been shown that early treatment with antiviral drugs in influenza reduces mortality, especially in adults (10), we observed a very high mortality of 61%, despite antiviral therapy. Whether influenza A H1N1 has unique properties that predispose to IAA remains to be investigated.

In a previous study, corticosteroid treatment was suggested to be the main risk factor for developing IAA in the ICU (1). Although 18 of 23 patients received steroids, our cohort also included 5 patients who did not receive corticosteroids, and 2 of them had no underlying condition. This suggests that corticosteroids might not be the only predisposing factor contributing to IAA.

Our study indicates that increased awareness of IAA as an early complication of influenza, prompt diagnoses, and initiation

Author Contributions: Study design: F.L.v.d.V., E.K., J.v.D., and P.E.V.; information technology and database: P.P.A.L.; data collection: F.L.v.d.V., E.K., C.J.H., B.J.A.R., J.v.P., P.-J.H., C.O.d.S., G.A.K., D.C.J.J.B., K.v.D., H.G.v.d.H., and P.E.V.; data analysis: F.L.v.d.V., E.K., P.P.A.L., and P.E.V.; statistical analysis: A.F.J.d.H.; writing of first draft: F.L.v.d.V., E.K., and P.E.V.; and revision of manuscript: all authors.

Originally Published in Press as DOI: 10.1164/rccm.201612-2540LE on April 7, 2017

Table 1. Underlying Disease, Influenza Type, *Aspergillus* Diagnosis, Initial Antifungal Therapy, and Outcome of 23 Patients with Influenza-associated Aspergillosis

Patient ID	Sex/ Age (*yr*)	Underlying Disease	Influenza Type	BAL GMI	Serum GMI	BAL Culture	*In Vitro* Susceptibility*	Corticosteroids	Initial Antifungal Therapy	Outcome†
2-1	F/34	None	A, H1N1		5.3		Wild-type and azole resistant	No	Voriconazole	Died (+27)
3-1	M/63	Hypertension, arthrosis	A, NT	1.5		*A. fumigatus*	Wild-type	Yes	Voriconazole + Lip-AmB	Died (+38)
3-3	M/65	FSGS	A, NT	6.5		*A. fumigatus*	Wild-type	No	Lip-AmB	Died (+21)
4-2	M/52	None	A, H1N1	13.6		*A. fumigatus*	Azole resistant	Yes	Voriconazole	Died (+13)
4-3	M/62	Churg-Strauss syndrome	A, NT	4.5	0.2	Negative		Yes	Voriconazole	Died (+12)
4-4	F/61	Kidney transplant	A, NT	11.7		*A. fumigatus*	Wild-type	Yes	Voriconazole	Died (+7)
5-3	M/62	None	A, NT		2.4			Yes	Voriconazole	Died (+11)
5-4	F/67	GPA	A, H1N1	8.3		*A. fumigatus*	Wild-type	Yes	Voriconazole + anidulafungin	Died (+8)
5-5	F/38	None	A, H1N1	2.0	0.9	*A. fumigatus*	Wild-type and azole resistant	Yes	Voriconazole	Died (+16)
5-6	M/53	None	A, NT	7.4	0.1	Negative		Yes	Voriconazole + caspofungin	Died (+43)
7-1	F/60	COPD	A, NT		2.8			Yes	Voriconazole	Died (+31)
7-2	F/67	Nonmalignant hematological disease	A, H1N1	2.8	0.1	Negative		Yes	Voriconazole	Died (+25)
7-3	M/66	GPA	A, NT	6.2	0.1	Negative		Yes	Voriconazole	Died (+27)
7-5	F/80	Churg-Strauss syndrome	A, H1N1		1.2		Wild-type	Yes	Lip-AmB	Died (+17)
2-2	F/53	Hematologic malignancy	A, H1N1	2.9	0.6	*A. fumigatus*	Wild-type	Yes	Voriconazole	Survived
2-3	F/45	Asthma, sinusitis	B	8.6	0.6	*A. fumigatus*	Wild-type and azole resistant	Yes	Voriconazole	Survived
4-1	M/64	None	A, H1N1	10.4		*A. fumigatus*	Wild-type	Yes	Voriconazole	Survived
5-2	M/50	COPD	B		0.6		Wild-type	Yes	Voriconazole	Survived
1-1	M/64	Myelofibrosis	A, NT	11.7		*A. fumigatus*		No	Voriconazole	Survived
6-1	M/70	Hematologic malignancy	A, H1N1	0.1	4.2	*A. fumigatus*	Wild-type	Yes	Voriconazole	Survived
6-2	M/57	Cystic fibrosis	A, H1N1	1.5		*A. fumigatus*	Wild-type	Yes	Voriconazole	Survived
6-3	M/55	None	A, H1N1	2.8		*A. fumigatus*		No	Voriconazole	Survived
7-4	M/65	Kidney transplant	A, NT	1.4	0.6	*A. fumigatus*		No	Voriconazole + micafungin	Survived

Definition of abbreviations: *A. fumigatus* = *Aspergillus fumigatus*; BAL = bronchoalveolar lavage; COPD = chronic obstructive pulmonary disease; FSGS = focal segmental glomerulosclerosis; GMI = galactomannan index; GPA = granulomatosis with polyangiitis; ID = identification number; Lip-AmB = lipid formulation of amphotericin B; NT = not typed.

*In three patients, wild-type and azole-resistant *A. fumigatus* colonies were recovered from culture.

†Parentheses show number of days from diagnosis of influenza.

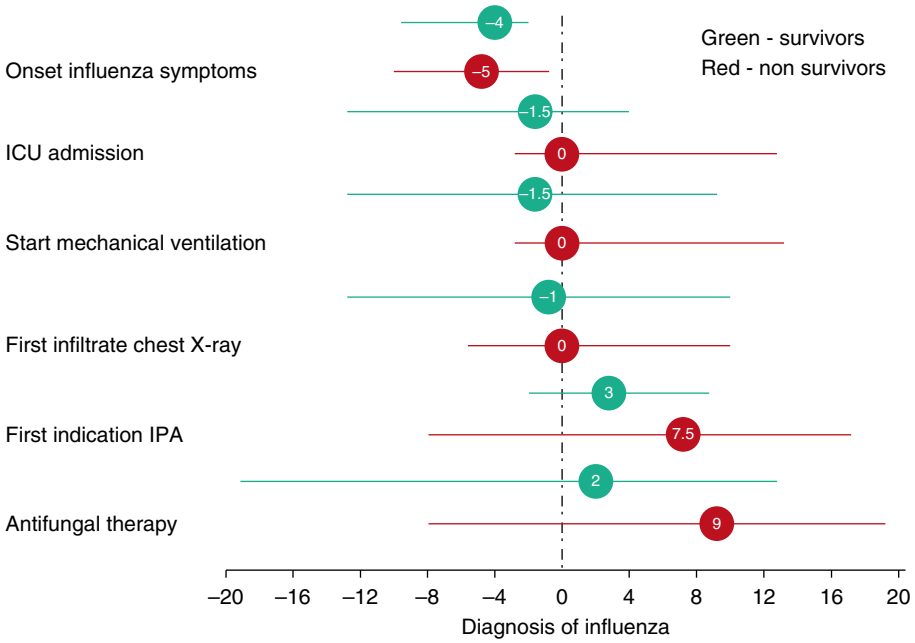

Figure 1. The median and range time to event from influenza diagnosis for survivors and nonsurvivors. The *x-axis* shows the number of days from diagnosis of influenza, with confirmed diagnosis at Day 0. ICU = intensive care unit; IPA = invasive pulmonary aspergillosis.

of appropriate antifungal therapy might prove to be important to decrease mortality of influenza in coming seasons. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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References

1. Wauters J, Baar I, Meersseman P, Meersseman W, Dams K, De Paep R, Lagrou K, Wilmer A, Jorens P, Hermans G. Invasive pulmonary aspergillosis is a frequent complication of critically ill H1N1 patients: a retrospective study. *Intensive Care Med* 2012;38:1761–1768.
2. Garcia-Vidal C, Barba P, Arnan M, Moreno A, Ruiz-Camps I, Gudiol C, Ayats J, Ortí G, Carratalà J. Invasive aspergillosis complicating pandemic influenza A (H1N1) infection in severely immunocompromised patients. *Clin Infect Dis* 2011;53:e16–e19.
3. Lat A, Bhadelia N, Miko B, Furuya EY, Thompson GR III. Invasive aspergillosis after pandemic (H1N1) 2009. *Emerg Infect Dis* 2010;16:971–973.
4. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, *et al*.;

European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Revised definitions of invasive fungal disease from 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 Group. *Clin Infect Dis* 2008;46:1813–1821.
5. Alshabani K, Haq A, Miyakawa R, Palla M, Soubani AO. Invasive pulmonary aspergillosis in patients with influenza infection: report of two cases and systematic review of the literature. *Expert Rev Respir Med* 2015;9:89–96.
6. Pagano L, Akova M, Dimopoulos G, Herbrecht R, Drgona L, Blijlevens N. Risk assessment and prognostic factors for mould-related diseases in immunocompromised patients. *J Antimicrob Chemother* 2011;66: i5–i14.
7. Kolwijck E, van der Hoeven H, de Sévaux RG, ten Oever J, Rijstenberg LL, van der Lee HA, Zoll J, Melchers WJ, Verweij PE. Voriconazole-susceptible and voriconazole-resistant *Aspergillus fumigatus* coinfection. *Am J Respir Crit Care Med* 2016;193:927–929.
8. Lee JY, Joo EJ, Yeom JS, Song JU, Yim SH, Shin DS, Yu JH, Ju DY, Yim JW, Song YS, *et al*. Aspergillus tracheobronchitis and influenza A co-infection in a patient with AIDS and neutropenia. *Infect Chemother* 2014;46:209–215.
9. van Paassen J, Russcher A, in 't Veld-van Wingerden AW, Verweij PE, Kuijper EJ. Emerging aspergillosis by azole-resistant *Aspergillus fumigatus* at an intensive care unit in the Netherlands, 2010 to 2013. *Euro Surveill* 2016;21(30):ii=30300.
10. Muthuri SG, Venkatesan S, Myles PR, Leonardi-Bee J, Al Khuwaitir TS, Al Mamun A, Anovadiya AP, Azziz-Baumgartner E, Báez C, Bassetti M, *et al*.; PRIDE Consortium Investigators. Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted to hospital with influenza A H1N1pdm09 virus infection: a meta-analysis of individual participant data. *Lancet Respir Med* 2014;2: 395–404.

Vascular Stiffness and Mechanotransduction: Back in the Limelight

To the Editor:

Decades of extensive research in the field of pulmonary hypertension (PH) have led to the discovery of several pathogenic drivers and, thus, targeting possibilities. Despite that, there remains a lack of effective therapeutic options with major vasodilation therapies that have a limited influence on vascular remodeling (1). Vascular remodeling, characterized by medial wall thickening, plexiform lesions, and intimal hyperplasia, is an outcome of excessive proliferation, migration, and survival of pulmonary artery smooth muscle cells (PASMCs), endothelial cells, and fibroblasts (2). This therapeutic deficit demonstrates a need for intensive investigation of the detailed mechanisms underlying vasodilation and vascular remodeling and, preferably, determining a link between them to target them together. Although vascular stiffening associated with aberrant collagen and elastin deposition in the extracellular matrix (ECM) at end-stage PH has been long recognized (3), emerging evidence also supports the idea that stiffening can precede the development of PH and promotes pulmonary vascular remodeling (4, 5). Vascular stiffness in the proximal and distal pulmonary arterial tree occurs in various forms of PH (3, 6), and stiffness is an index of disease progression (7). Furthermore, recent studies demonstrate that pulmonary vascular (PV) stiffness has significant prognostic value in PH. PV stiffness correlates with mortality in patients with PH, and moreover, measurements of PV stiffness are considered to be more accurate in assessing right ventricular afterload and may even be superior to PV resistance in predicting mortality (8, 9). These observations raise the possibility that PV stiffness is a critical factor that must be treated to improve outcomes for patients with PH.

However, the exact role of vascular stiffening in the development and progression of PH has yet to be defined. In particular, the field has lacked a clear understanding of the spatiotemporal development of PV stiffness and what distinct responses to proximal versus distal PV stiffening contribute to PH pathogenesis and progression. In addition, although it is becoming increasingly clear that measurement of changes in PV stiffness/arterial flow pulse waves are likely to be incorporated into medical practice for diagnosis or prognosis of PH, many remain skeptical that vascular stiffness is a desirable treatment target. A major contributor to this skepticism is the lack of studies delineating a causal relationship between artery stiffness and PH progression.

An essential question that further arises is what exactly leads to an increase in distal vascular stiffness in response to pathological stimuli. In parallel, we do not know how early changes in the local mechanical environment contribute to progressive vascular remodeling and promote the development of PH. What are the mechanosensitive pathways/factors that are activated, and how can they regulate cellular proliferation, survival, metabolism, and the ECM, particularly during the development and progression of PH?

Three recent and independent studies shed light on these important issues. Together, Liu and colleagues (5) and Bertero and colleagues (10) suggest that vascular stiffness–induced mechanical stress is sufficient to activate cellular pathways in vascular cells, leading to enhanced proliferation, migration, and matrix deposition. These changes can, in turn, further perpetuate vascular stiffness, giving rise to a self-sustainable loop amplifying vascular remodeling in PH. Ruffenach and colleagues (11) provide strong support in favor of microRNA(miR)-204/Runt-related transcription factor 2 (RUNX2)/hypoxia-inducible factor-1α axis as a driver of stiffness via promoting vascular calcification in distal vasculature (Figure 1).

Human PH patients and animal models for PH, such as hypoxic calves, rats, and mice, are all characterized by increased stiffness or reduced compliance of pulmonary arteries (PAs) and/or increased PA impedance (6, 12, 13). Considerable work has been invested in understanding large vessel stiffening at the macroscopic level; however, little is known about distal wall stiffening. Liu and colleagues (5), taking the lead in this direction, demonstrated that PA stiffening arises in the distal vasculature, followed by stiffening of more proximal vessels in two rat models of PH: monocrotaline (MCT) and Su5416/hypoxia. This stiffening took place early in the course of experimental PH, preceding alterations in hemodynamics or right ventricular dysfunction. This strongly suggested a causative role of vascular stiffening in the pathogenesis of PH. These findings were supported by a significant increase in small vessel stiffness observed in patients with idiopathic pulmonary arterial hypertension. Further cementing this role, the authors showed that matrix stiffening directly activated the proliferation of PASMCs and pulmonary artery endothelial cells and triggered PASMCs to produce ECM and exaggerated traction forces. On the basis of their