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SUPPLEMENT ARTICLE

Invasive Pulmonary Aspergillosis in Adults With Avian Influenza A (H7N9) Pneumonia in China: A Retrospective Study

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Cases of severe influenza with *Aspergillus* infection are commonly reported in patients with severe influenza. However, the epidemiology, risk factors, and outcomes of invasive pulmonary aspergillosis (IPA) in patients with avian influenza A (H7N9) infection remain unclear. We performed a retrospective multicenter cohort study. Data were collected from patients with avian influenza A (H7N9) infection admitted to 17 hospitals across China from February 2013 through February 2018. We found that IPA was diagnosed in 18 (5.4%) of 335 patients; 61.1% of patients with IPA (11 of 18) were identified before or within 2 days after an H7N9 virus–negative result. The median hospital stays in patients with or without IPA were 23.5 and 18 days, respectively ($P < .01$), and the median intensive care unit stays, respectively, were 22 and 12 days ($P < .01$). Smoking in the past year and antibiotic use for >7 days before admission were independently associated with IPA (adjusted odds ratio [95% confidence interval], 6.2 [1.7–26] for smoking and 4.89 [1.0–89] for antibiotic use). These findings provided important insights into the epidemiology and outcomes of IPA in patients with H7N9 infection in China.

Keywords. avian influenza A; H7N9; invasive pulmonary aspergillosis.

Since 2013, avian influenza A (H7N9) has persisted through 5 epidemic seasons in China, during annual winter-spring epidemic waves, resulting in mortality rates of ≥39% [1, 2]. The age distribution of H7N9 avian influenza is similar to that for seasonal influenza; that is, most patients are >65 years old, but cases shifted from the elderly to middle-aged adults, and from urban to semiurban and rural areas [2]. However, owing to the general lack of immunity in the population and delayed diagnosis, H7N9 avian influenza has become a serious disease, with 76.6% of patients requiring admission to the intensive care unit (ICU) [3]. In contrast, the ICU admission risk for other influenzas is only 15%–19% [4].

Invasive pulmonary aspergillosis (IPA) may occur in the setting of severe influenza infection. Such cases of influenza and IPA coinfection have been reported with increasing incidence since 1979 [5–7]. A recent European cohort study confirmed that influenza itself was an independent risk factor for IPA. The incidence of *Aspergillus* infection in patients in the ICU with severe influenza is as high as 19%. Conversely, IPA developed in only 5% of patients with severe community-acquired pneumonia who had a negative polymerase chain reaction test result for airway influenza [8]. However, it is unclear whether the risks of IPA and death are higher in patients with avian influenza A (H7N9) infection than in those with other types of influenza. Accordingly, in the current study, we collected clinical data for patients with avian influenza A (H7N9) infection from 17 hospitals in China and described the epidemiology, clinical characteristics, risk factors, and outcomes of IPA in these patients.

METHODS

Study Population

A retrospective analysis was performed in patients with avian influenza A (H7N9) admitted to 17 hospitals in China from February 2013 to February 2018, over 5 consecutive influenza seasons. The end point of our study was discharge from the hospital with full recovery or death. The case definitions of confirmed human infection with the novel H7N9 virus were described by Gao et al [3]. Case definitions of IPA followed the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium [9], and only patients in whom IPA was proved or probable were enrolled in our study. *Aspergillus* isolates in respiratory samples but absence of newly pulmonary infiltrates was described as colonization, and cases with such findings were excluded. We used culture and microscopic morphological characteristics to distinguish different types of *Aspergillus*.

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Data Collection

Clinical and laboratory data were retrospectively extracted from the original medical records. The following data were collected: sex, age, date of onset of symptoms, underlying diseases, secondary bacterial and fungal infections, length of hospital stay and length of ICU stay (both in days), complications, clinical and laboratory features, mortality rate, chest computed tomography scan results, antiviral therapy, antifungal therapy, antibiotics (including name and duration of administration before admission), and corticosteroid therapy (including duration, daily and cumulative doses before general microbe testing or fungal culture).

Statistical Analysis

Continuous variables were summarized as either medians with interquartile ranges (IQRs) or means with standard deviations. For categorical variables, we calculated the percentages of patients in each category. We used χ^2 tests, independent-group Student *t* tests, or Fisher exact tests, as appropriate, to compare clinical characteristics between the subgroups of patients who had IPA and those who did not. We used multiple logistic regression analysis to identify independent predictors of IPA and death. Results with *P* values <.05 were considered statistically significant. All analyses were performed with the use of SPSS software for Windows (release 18.0).

RESULTS

Clinical Characteristics of Patients With Avian Influenza A (H7N9) Infection

From February 2013 to February 2018, a total of 335 patients with confirmed H7N9 infection (232 men and 103 women) were admitted to the hospitals. Their median age was 57 years (IQR, 45–68 years); 148 patients (44.2%) were >60 years old. Moreover, 194 patients (57.9%) had underlying diseases. The most common underlying diseases were hypertension (133 cases [39.7%]) and diabetes (57 cases [17%]). At the time of admission, acute respiratory distress syndrome (ARDS) had been diagnosed in 226 patients (67.5%). Another 18 cases gradually progressed to ARDS, accounting for 72.8% of all patients. In addition, shock developed in 108 patients (32.2%) , 225 patients (67%) were admitted to the ICU, and 96 (28.7%) died. The median hospital stay in surviving patients was 18 days (IQR, 12–29 days).

Of the 335 patients with H7N9 infection included in this study, 18 (5.4%) had probable IPA diagnosed. Twelve patients with IPA were admitted to the ICU (5.3% [12 of 225 admitted to the ICU]), and 6 were not (5.5% [6 of 110 not admitted to the ICU]). IPA was diagnosed a median of 17 days (range, 9–39 days) after onset of avian influenza A H7N9 symptoms and a median of 9.5 days (5–16 days) after admission to the ICU (Table 1). Eleven patients had IPA diagnosed before or within 48 hours after an H7N9 virus–negative result was obtained, and another 7 had IPA diagnosed after >48 hours after a virus-negative result was obtained. Except for 1 case of *Aspergillus flavus*, all other cases were *A. fumigatus*. There were 12 cases of IPA from 1 center in different years (First Affiliated Hospital of Zhejiang University; 8.1% [12 of 147]), and 6 cases from the other 3 hospitals (3.2% [6 of 188]). There were no significant differences in underlying diseases (hypertension, diabetes, heart disease, chronic obstructive pulmonary disease [COPD], chronic nephropathy, and malignant tumors) between the *Aspergillus*-positive and *Aspergillus*-negative groups. However, there was a significant difference in smoking status in the past year ($P < .05$).

Complications and Treatment in the IPA and Non-IPA Groups

There were no significant differences in ARDS, shock, liver function damage, and acute renal injury between the *Aspergillus*-positive and *Aspergillus*-negative groups. However, patients with IPA were more likely to have secondary bacterial infection (61.1% vs 31.2%, respectively; $P < .01$), including pulmonary (61.1% vs 30%; $P < .01$), blood stream, and thoracic infection (Table 1).

More than 50% of patients in the IPA and non-IPA groups required mechanical ventilation support (55.6% vs 50.8%, respectively), and some required extracorporeal membrane oxygenation (22.2% vs 11.4%). Most patients in the 2 groups used glucocorticoids (83.3% vs 74.4%, respectively). The median durations of glucocorticoid treatment were 6.5 (IQR, 4–11.5) and 7 days (3–11 days) days, respectively. There were no significant differences between the 2 groups. However, patients with IPA were more likely to use hormones at the maximal daily dose than those without IPA (median [IQR] methylprednisolone equivalent dosage, mg/d, 80 [40–120] vs 80 [40–80] mg/d; $P < .01$).

Clinical Outcomes in the IPA and Non-IPA Groups

The mortality rates in patients with or without IPA were high (22.2% vs 29%; $P > .05$). The median time from admission to death, the median hospital stay, and the median ICU stay in patients with IPA were significantly longer than those in patients without IPA (median [IQR], 33 [18.75–51.25] vs 12.5 [5–24.25] days, 23.5 [16.25–45.25] vs 18 [11–29] days, and 22 [16.25–57.5] vs 12 [6–26] days, respectively; $P < .01$) (Table 1).

Analysis of Risk Factors for the Development of IPA

Multivariate analysis showed that smoking in the past year and antibiotic use for >7 days starting before admission were independent risk factors for IPA in patients with avian influenza A (H7N9) infection (odds ratio, 6.2 [95% confidence interval, 1.7–26] and 4.89 [1.0–89], respectively), whereas age, sex, ICU stay, mechanical ventilation, and glucocorticoid use were not independent risk factors for IPA ($P < .001$; Table 2)

DISCUSSION

In the current study, the epidemiology, risk factors, and outcomes of IPA infection in patients with avian influenza

Table 1. Demographic Characteristics, Underlying Diseases, and Clinical Characteristics of Patients With Avian Influenza With or Without *Aspergillus* Infection

Variable	*Aspergillus* Positive (n = 18)	*Aspergillus* Negative (n = 317)	*P* Value
Demographics			
Age, mean (SD), y	60.56 (12.69)	55.82 (16.23)	.16
Male sex, no. (%)	12 (66.7)	220 (69.4)	.80
Underlying disease, no. (%)			
Any	12 (66.7)	182 (57.4)	.13
Hypertension[a]	7 (41.2)	126 (39.7)	>.99
Diabetes mellitus[a]	4 (23.5)	53 (16.7)	.50
Heart disease[a]	2 (11.8)	30 (9.5)	.67
COPD[a]	2 (11.8)	14 (4.4)	.19
Cancer[a]	0	14 (4.4)	>.99
Immunodeficiency[a]	0	5 (1.6)	>.99
Chronic kidney disease[a]	0	12 (3.8)	.78
Pregnancy[a]	0	7 (2.2)	>.99
Smoking in the past 1 y, n (%)	9 (50)	67 (21.1)	.007
Complications, no. (%)			
ARDS	15 (83.3)	211 (66.6)	.19
Shock	8 (44.4)	100 (31.5)	.19
Liver damage	11 (61.1)	197 (62.1)	.46
Acute kidney injury[a]	4 (22.2)	47 (14.8)	.53
Secondary bacterial infection	11 (61.1)	99 (31.2)	<.01
Sputum or BAL fluid	11 (61.1)	95 (30)	<.01
Blood	7 (38.9)	56 (17.7)	.053
Hydrothorax[a]	1 (5.6)	11 (3.5)	.85
Treatment, no. (%)			
Mechanical ventilation	10 (55.6)	161 (50.8)	.89
Extracorporeal membrane oxygenation[a]	4 (22.2)	36 (11.4)	.15
Corticosteroid treatment	15 (83.3)	236 (74.4)	.58
Duration of therapy, median (IQR), d[b]	6.5 (4–11.5)	7 (3–11)	.23
Maximum methylprednisolone equivalent dosage, median (IQR), mg/d[b]	80 (40–120)	80 (40–80)	<.01
Administration of antiviral treatment	18 (100)	313 (98.7)	>.99
Duration from symptoms to NAI, median (IQR), d[b]	7 (5.25–8.75)	7 (5–9)	.71
Duration of viral shedding, median (IQR), d[b]	7 (5–8)	7 (5–10)	<.01
Administration of antibiotic treatment for >7 d, no./total (%)	17/18 (94.4)	183/249 (73.5)	.047
Clinical outcome			
Death, no. (%)	4 (22.2)	92 (29)	.79
Time from admission to death, median (IQR), d[b]	33 (18.75–51.25)	12.5 (5–24.25)	<.01
Discharge from hospital, no. (%)	14 (77.8)	225 (71)	.79
Length of stay in hospital stay, median (IQR), d[b]	23.5 (16.25–45.25)	18 (11–29)	<.01
Length of ICU stay, median (IQR), d[b]	22 (16.25–57.5)	12 (6–26)	<.01
Duration of glucocorticoid treatment, median (IQR), d[b]	6.5(4–11.5)	7 (3–11)	.79

Abbreviations: ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; IQR, interquartile range; NAI, neuraminidase inhibitor; SD, standard deviation.

[a]Compared using Fisher exact test.

[b]Compared using Mood median test.

A (H7N9) were described and analyzed for the first time. Our results showed that patients with avian influenza A (H7N9) complicated with IPA had prolonged hospital and ICU stays. Smoking in the past year and antibiotic use for >7 days starting before admission were independent risk factors for IPA infection in patients with avian influenza A H7N9.

Previous studies of IPA in patients with influenza have evaluated relationships with influenza A H1N1 and influenza B [5–8] but not avian influenza A (H7N9). In our current study, we found that the incidence of IPA in patients admitted to the ICU with avian influenza A (H7N9) was obviously lower than that in patients admitted to the ICU with influenza A H1N1 or influenza B infection (5.4% vs 19%) [5–8]. Instead, the incidence of *Aspergillus* infections in our study was similar to that of noninfluenza community-inquired pneumonia or other critical illnesses in patients in the ICU [8]. The lack of bronchoalveolar lavage galactomannan measurement in our study may have resulted in an underestimation of the incidence of IPA. However,

Table 2. Risk Factors for the Development of Invasive Pulmonary Aspergillosis

Variable	OR (95% CI)
Age	1.0 (.9–1.1)
Sex	0.3 (.7–1.3)
ICU admission	1.2 (.3–5.9)
Smoking in past 1 y	6.2 (1.7–26)
Underlying disease	4.2 (.7–8.2)
Corticosteroid treatment	1.2 (.3–6.1)
Mechanical ventilation	0.7 (.1–3.3)
Extracorporeal membrane oxygenation	2.2 (.3–1.3)
IVIG	1.5 (.4–5.8)
Corticosteroid dosage, mg/d	
≤40	1
40–80	1.04 (.29–3.56)
80–120	0.95 (.05–6.07)
120–160	0.98 (.14–4.49)
>160	1.47 (.07–9.77)
Corticosteroid duration, d	
≤5	1
>5	0.65 (.22–1.97)
Antibiotic duration	
≤7	1
>7	4.89 (1.0–89)

Abbreviations: CI, confidence interval; ICU, intensive care unit; IVIG, intravenous immunoglobulin; OR, odds ratio.

these differences could also be explained by the fact that previous studies have been based on comprehensive data of ICU infections.

In China, owing to government regulations, the management of H7N9 avian influenza A is similar to that of severe ARDS infection. Patients with confirmed avian influenza A (H7N9) infection should stay in designated hospitals in a single isolation ward or in a negative-pressure isolation ward, which may reduce *Aspergillus* exposure and avoid cross-infection between different patients because inhalation of fungal spores is the most common portal of entry. These physical isolation measures may alter the natural history of IPA in patients with avian influenza A (H7N9). Moreover, for patients with severe influenza, single isolation not only prevents the spread of influenza but also reduces the risk of *Aspergillus* infection in the patients themselves.

Our multivariate analysis of risk factors showed that smoking in the past year and long-term antibiotic use (>7 days) were independent risk factors for IPA. These factors commonly contribute to the colonization of *Candida albicans* but are not risk factors for *Aspergillus* infection. However, some studies have shown that previous use of antibiotics significantly increases the risk of IPA in patients with COPD [10, 11]. In addition, smoking is the most common risk factor for COPD. Therefore, it is feasible that smoking in the past year induced injury to respiratory epithelial cells and affected bronchomucosal clearance, which may facilitate the colonization and later invasion of *Aspergillus*.

Our study also had some limitations. First, it was a retrospective study; therefore, we could not use more accurate methods to confirm the diagnosis of IPA. Owing to the seriousness of H7N9 avian influenza infection, we were not able to obtain evidence of a histological diagnosis. Therefore, our study included only clinically diagnosed cases, which may have led to omission of some patients with IPA. Second, we did not select the control group from the same period in the same area, and the obtained data were all compared with foreign data, limiting the scientific reliability of the conclusions. Notably, it is difficult to encounter critically ill patients with pneumonia who have no infectious diseases but have to be admitted to the isolation ward. Third, as a retrospective study, some data were missing, such as the daily dosage, specific time, and duration of glucocorticoid treatment before ICU admission. Therefore, the effects of glucocorticoid usage on IPA cannot be fully evaluated. Accordingly, we were not able to accurately compare our results with those of a previous study in which glucocorticoid use was found to be independently associated with IPA [8].

In conclusion, IPA could develop in patients infected with avian influenza H7H9, leading to prolonged hospital and ICU stays. Smoking in the past year and antibiotic use for >7 days were independent risk factors for IPA. Our results also suggested that patients with H7N9 avian influenza should avoid overuse of antibiotics and that those who have smoked within the past year should be evaluated for *Aspergillus* infection.

Notes

Author contributions. H. G. and L. L. designed and conceptualized the study. P. Z., C. W., and S. Z. designed the table to collect the data and analyzed the data. F. G., L. Y., Y. Z., P. L., Y. S., Y. W., and X. Z. provided clinical data. P. Z., C. W., and H. G. wrote the manuscript. L. T. helped revise the manuscript. All authors have read and approved the final manuscript.

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