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Clinical study of invasive pulmonary aspergillosis following influenza A H1N1

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Abstract

This study to analyze the clinical characteristics of patients with invasive pulmonary aspergillosis (IPA) following influenza A (H1N1) infection.

We retrospectively analyzed 10 cases with IPA following H1N1 infection. The clinical manifestations, laboratory examination results, chest computed tomography, and treatments were analyzed.

Clinical manifestations: all 10 cases had typical flu-like symptoms at the onset of the disease, among which 7 patients developed dyspnea in the late stage, and 8 patients had hemoptysis. Laboratory examination: the absolute and percentage of peripheral blood lymphocytes in all 10 patients were declined, among which 5 cases were with decreased CD3^{+} CD4^{+} T cells/lymphocytes; 9 cases with increased bronchoalveolar lavage fluid galactomannan; 6 cases with increased serum galactomannan; 1 case with bronchoalveolar lavage fluid cultured aspergillus fumigatus; and 2 cases with aspergillus by second-generation sequencing. Chest computed tomography: all patients showed multiple diffused ground-glass opacities at the beginning, along with linear or reticular interstitial changes. Two cases had multiple subarachnoid nodules with halo signs, 3 cases had consolidation in multiple segments of both lungs, 2 cases had cavities, and 4 cases were with pleural effusion. Treatment: 10 patients were treated with antiviral and anti-Aspergillus drugs after admission. Four patients received respiratory support. All 10 cases were cured and discharged.

Early diagnosis of IPA in influenza A (H1N1) patients is the key to successful treatment.

Abbreviations: ARDS = acute respiratory distress syndrome, IPA = invasive pulmonary aspergillosis.

Keywords: alveolar lavage fluid, anti-Aspergillus treatment, galactomannan, H1N1, invasive pulmonary aspergillosis

1. Introduction

Influenza is a common respiratory infectious disease caused by viral infection. In 1918, the influenza caused about 50 million deaths worldwide, making it the most lethal disease in human history. In early 2009, a new influenza A (H1N1) strain was first found in Mexico and quickly spread to the world. The first case of H1N1 in China was found in April of that year and after that, H1N1 broke out in May of the same year. The incidence of influenza A (H1N1) is seasonal, and the epidemic period is from October to March of the following year.[1] In general, influenza A without serious complications is self-limiting.[2,3] However, a small number of patients have very serious symptoms, including bacterial and fungal pneumonia, respiratory distress and hypoxemia, and even death.

Typical invasive pulmonary aspergillosis (IPA) usually occurs in immunodeficient hosts. However, Aspergillus infections detected in non-immunodeficiency hosts are often considered colonization.[2,4] The 6-week mortality rate of patients with IPA is 20% to 30%,[1,5] which is higher than that of patients with critical illness.[1,6] In 1952, studies first reported the detection of Aspergillus in the lung tissue of influenza patients. Cases of influenza with Aspergillus infection were occasionally reported decades ago and only a few small samples of clinical trials have been reported in the past 5 to 10 years.[4,7,8] Influenza virus infection has become an independent factor in IPA.[9]

In this study, by analyzing the epidemiological and prognostic results of patients with IPA after influenza A (H1N1) infection, we investigated whether there is an independent correlation between influenza A (H1N1) and IPA.

2. Materials and methods

2.1. Ethics

Prior written and informed consent were obtained from every patient and the study was approved by the ethics review board of Kunming Second People's Hospital.

2.2. Patients

This is a retrospective study. We recruited 47 patients diagnosed with H1N1 influenza admitted to the Department of Respiratory

and Critical Care of the Second People's Hospital of Kunming. They were hospitalized from October 2018 to February 2019. All 47 patients underwent influenza test for H1N1 influenza virus and galactomannan (GM) antigen test for Aspergillus Infection. All patients were diagnosed with positive H1N1 influenza virus by a nucleic acid test that was performed on the day of admission. The diagnostic criteria were based on the "Influenza Treatment Program (2018 Edition)." [10] Based on patient's medical history, clinical signs, and chest computed tomography (CT) examination results, as well as the high risk factors of Aspergillus infection, all patients were routinely subjected to GM antigen test for further analysis of IPA based on the IDSA Clinical Practice Guidelines: Diagnosis and Management of Aspergillosis (2016 Edition).[3] Aspergillus infection was diagnosed by histopathological/ cytological examination and the strain subtypes were identified by microbial culture. The clinical diagnostic criteria for Aspergillus included presence of GM in serum and bronchoalveolar lavage fluid (BALF). GM > 0.5 was used as standard. Exclusion criteria were: Patients with influenza A-negative pneumonia; Patients with noninfectious pneumonia, such as pulmonary edema, atelectasis, pulmonary interstitial disease, pulmonary vascular inflammation, lung cancer, etc; Simple IPA infection without H1N1 influenza; Patients <14 years old.

2.3. Data collection

The clinical manifestations, laboratory examination, chest CT findings, treatment, and outcomes of patients were retrospectively analyzed and discussed.

2.4. Statistical analysis

The data were analyzed using SPSS software. The measurement data is expressed as mean±standard deviation (SD), and analyzed by t test. The count data is expressed as a percentage (%), and compared using the χ^2 test. $P < .05$ indicates significant difference.

3. Results

3.1. The characteristics of patients

Clinical data of patients were shown in Table 1. A total of 47 patients with H1N1 were treated, of whom 10 were with IPA. The incidence of IPA following H1N1 was 21.3%. The IPA patients included 9 males and 1 female, and they were all from Kunming. The ratio of male patients in the H1N1 patients with IPA was significantly higher than that in those without IPA

($P < .05$). The age ranged from 28 to 67 years with a median of 51 years (3 cases > 65 years). The time ranged from 5 to 7 days from onset to hospitalization, with a median of 5 days. The time that from onset to diagnosis of influenza A H1N1 pneumonia was 4 to 8 days, with a median of 6 days. The time that from diagnosis of influenza A H1N1 pneumonia to suspected IPA was 1 to 6 days, with a median of 3.5 days. The time from suspected IPA to diagnosis of IPA was 2 to 5 days, with a median of 2 days. The underlying diseases of the patients included: 3 cases with diabetes, 3 cases with hypertension, 2 cases with chronic obstructive pulmonary disease, 8 cases with hyperlipidemia, 2 cases with diabetes, hypertension and hyperlipidemia, and 1 case with diabetes, hypertension, hyperlipidemia, and chronic obstructive pulmonary disease. H1N1 patients with IPA had significantly higher percentage of hyperlipidemia than those without IPA ($P < .05$). In addition, 8 cases had smoking history, 2 cases had body mass index (BMI) ≥ 30, 6 cases had 26 ≤ BMI < 30, and 2 cases had 22 ≤ BMI < 26.

3.2. Clinical symptoms

The clinical symptoms of patients were shown in Table 2. Ten patients had fever, cough, and body aches in the early stage of the disease. Three cases were accompanied by chills. Seven cases had wheezing, 8 cases had hemoptysis, and 1 case had chest pain.

3.3. Blood test after diagnosis of influenza virus pneumonia

There were 2 patients with increased peripheral blood leukocytes, neutrophils, and procalcitonin. Ten patients had decreased lymphocyte count. Eight patients had decreased absolute lymphocyte numbers. Ten patients had increased hypersensitive C-reactive protein. Seven patients showed a decrease in the CD3+ CD4+ T cell/lymphocyte ratio, indicating a decrease in immune function in patients with influenza virus pneumonia. The biochemical index was compared between H1N1 patients with IPA and those without IPA. As shown in Table 3, the absolute lymphocyte numbers and the lymphocyte percentage of H1N1 patients with IPA were significantly lower than those without IPA ($P < .05$). H1N1 patients with IPA and without IPA were not significantly different in other biochemical indexes.

3.4. Blood test after diagnosis of IPA

There were 3 patients with increased peripheral blood leukocytes, and procalcitonin. Five cases had decreased peripheral blood lymphocytes. And hypersensitive C-reactive protein was in-

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Table 1

Comparison of basic clinical information of H1N1 patients with or without secondary IPA.

	Male, n (%)	Age, years (mean±SD)	Body mass index (kg/m²) (mean±SD)	Underlying diseases, n (%)			
				Hypertension	Diabetes	Hyperlipidemia	Chronic obstructive pulmonary disease
H1N1 patients with secondary IPA (n=10)	9 (90%)	51.4±14	28.96±2.8	3 (30%)	3 (30%)	8 (80%)	2 (20%)
H1N1 patients without secondary IPA (n=37)	16 (43.2%)	51.4±15	25.67±3.7	8 (21.6%)	6 (16.2%)	8 (21.6)	5 (13.5%)
t		0.009	2.59				
P		.993	.013				
χ^2	5.162			0.018	0.281	9.490	0.000
P	.023			.893	.596	.002	.992

H1N1 = influenza A, IPA = invasive pulmonary aspergillosis.

Table 2

Symptoms of patients with secondary IPA.

Symptoms	Case/total cases/ratio
37.3 ≤ body temperature < 38.0℃	3/10/30%
38.0 ≤ body temperature < 39.0℃	5/10/50%
Body temperature ≥ 39.0℃	2/10/20%
Cough/ sputum	10/10/100%
Body aches	10/10/100%
Chill	3/10/30%
Wheezing	7/10/70%
Hemoptysis	8/10/80%
Hemoptysis ≤ 50 mL/d	6/8/75%
Hemoptysis >50 mL/d	2/8/25%
Chest pain	1/10/10%

IPA = invasive pulmonary aspergillosis.

Table 5

Specific GM test results of patients with secondary IPA.

NO.	BALF GM	Serum GM	BALF culture	mNGS
1	0.75	0.17		
2	2.11	0.53		
3	1.43	0.49		
4	6.52	3.84	(+) Aspergillus fumigatus	(+) Aspergillus
5	0.89	0.33		
6	8.13	0.96		
7	4.85	0.74	—	(+) Aspergillus
8	1.36	0.68		
9	0.41	0.11		
10	2.07	0.57		

BALF = bronchoalveolar lavage fluid, GM = galactomannan, mNGS = metagenomics next-generation sequencing.

creased in all patients. The proportion of CD3⁺ CD4⁺ T cells/ lymphocytes increased in 5 cases, indicating that the immune function of patients improved after anti-infective treatment. The biochemical index of H1N1 patients with IPA before and after treatment was compared. As shown in Table 4, the absolute lymphocyte numbers after treatment in H1N1 patients with IPA were significantly higher than those before treatment ($P < .05$). The other biochemical indexes showed no significant difference.

3.5. Pathogen test

As shown in Table 5, 9 cases showed an increase in BALF GM (range 0.79–8.13; median: 1.75; SD: 2.55). Serum GM was elevated in 6 patients (range 0.51–3.84; median: 0.55; SD: 1.03). As shown in Table 3, 1 case had Aspergillus fumigates in cultured BALF. Two cases were Aspergillus positive on Metagenomics Next-Generation Sequencing.

3.6. Chest imaging

On chest CT, the initial manifestations of 10 patients showed multiple diffuse ground-glass opacity with linear or reticular

interstitial changes in the lungs (Fig. 1A). Two cases showed a reticular change as disease progression, and multiple small nodules were visible near the pleura; 3 cases showed consolidation in multiple segments of both lungs; and 2 cases had cavity-like changes, and 4 cases had pleural effusion (Fig. 1, B and C).

3.7. Treatment

Anti-infection treatment: 10 patients started oral oseltamivir 4 to 7 days (median time 4.5 days) after the onset at a dose of 75 mg/ time, q12 hour. Five of them were treated with antiviral therapy for 7 days, 2 patients were treated for 10 days, and 3 patients were treated for 14 days. All 10 patients were treated with antibiotics intravenously after admission, of whom 6 were treated with moxifloxacin, 3 were treated with cefoperazone sulbactam, and 1 was treated with cefuroxime and azithromycin. The patient was given antifungal therapy as soon as possible after diagnosis/ clinical diagnosis of IPA (1–2 days, median treatment time was 1 day). The antifungal therapy was voriconazole (Pfizer, New York, USA), given intravenously at a dose of 400 mg on the first day, q12 h, 200 mg from the next day, q12 h. Eight of them were treated with voriconazole on the first day of clinical diagnosis and 2 on the second day of clinical diagnosis. Five patients were

Table 3

Comparison of biochemical index of H1N1 patients with or without secondary IPA (mean±SD).

	White blood cell number (×10⁹/L)	Neutrophil percentage (%)	Absolute lymphocyte number (×10⁹/L)	Lymphocyte percentage (%)	C-reactive protein (mg/L)	Procalcitonin (ng/mL)
H1N1 patients with secondary IPA (n=10)	7.21±3.14	70.39±11.3	0.89±0.28	13.68±4.30	53.93±31.07	0.4±0.4
H1N1 patients without secondary IPA (n=37)	5.84±2.66	64.46±14.48	1.26±0.54	24.62±12.04	35.93±27.7	0.2±0.15
t	1.396	1.196	−2.057	−4.555	1.778	1.563
P	.170	.238	.045	.000	.082	.150

H1N1 = influenza A, IPA = invasive pulmonary aspergillosis.

Table 4

Comparison of biochemical index of H1N1 patients with secondary IPA before and after treatment (mean±SD).

		White blood cell number (×10⁹/L)	Neutrophil percentage (%)	Absolute lymphocyte number (×10⁹/L)	Lymphocyte percentage (%)	C-reactive protein (mg/L)	Procalcitonin (ng/mL)
H1N1 patients with secondary IPA (n=10)	Before treatment	7.21±3.14	70.39±11.30	0.89±0.28	13.68±4.3	53.93±31.07	0.4±0.4
	After treatment	8.95±3.89	69.07±14.32	1.21±0.39	16.22±8.15	47.04±42.95	0.54±0.75
t		−1.83	0.320	−2.996	−1.273	0.883	−1.154
P		.100	.756	.015	.235	.400	.278

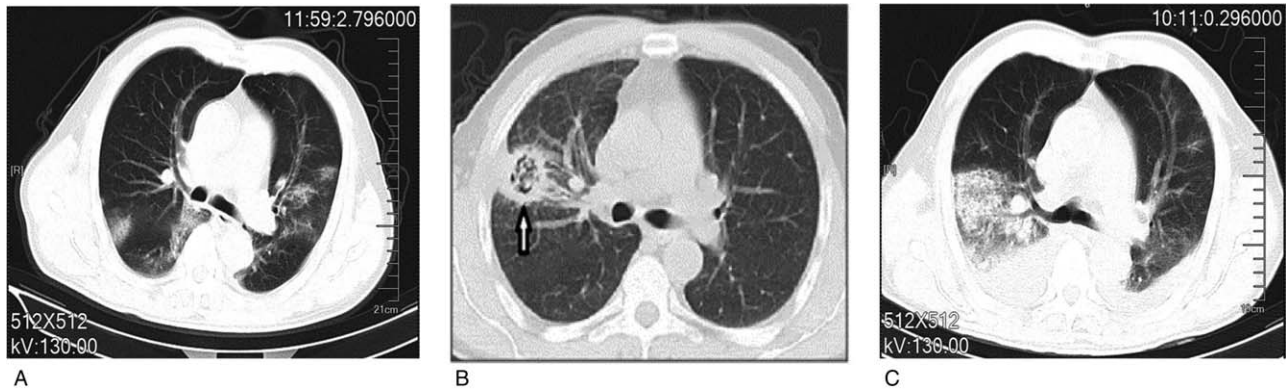H1N1 = influenza A, IPA = invasive pulmonary aspergillosis.

Figure 1. Chest image of H1N1 process in patients. A, Diffuse ground-glass opacity in both lungs. B, Consolidation with cavity formation in right lung. C, Multiple consolidation with pleural effusion in both lungs.

treated with oral voriconazole for 21 days after intravenous voriconazole for 7 days. Four patients were treated with oral voriconazole for 14 days after intravenous voriconazole for 14 days. One patient who was critically ill was treated with oral voriconazole for 21 days after intravenous voriconazole for 21 days. The average course of voriconazole intravenous + oral administration in 10 patients was 31.5 days.

Glucocorticoid therapy: 5 patients were treated with glucocorticoids for 1 to 3 days (median time 2 days) before admission. After admission, only 3 patients continued to receive glucocorticoid therapy for 4 to 7 days (median time 5.5 days).

Ventilation: 1 patient with mild-to-moderate Acute Respiratory Distress Syndrome (ARDS) received noninvasive ventilator-assisted breathing for 6 days; 3 patients with severe ARDS underwent mechanical ventilation with oral tracheal intubation for 3 to 5 days (median time 4 days).

Clinical outcome: 10 patients were all discharged from hospital after treatment. The total hospital stay in the hospital was 10 to 30 days (median time 18.5 days), and the treatment success rate was 100%.

4. Discussion

Epidemiological study has shown that influenza A viruses mainly attack young adults aged 25 to 45.[11] Secondary bacterial infection after influenza infection is the leading cause of death. Studies have shown that influenza-related pneumonia is mostly found in patients aged ≤65 years during influenza A outbreaks.[12,13] In this study, the median age of 10 patients with influenza A (H1N1) viral pneumonia was 51 years, and 3 patients were >65 years old. Male H1N1 patients and H1N1 patients with hyperlipidemia were more likely to have IPA.

Host defense against viruses mainly depends on T lymphocyte-mediated cellular immunity.[14] It is currently believed that lymphopenia is mainly caused by virus-induced apoptosis of T lymphocytes, which may lead to an increased risk of IPA in patients.[15–17] In particular, when lymphocyte percentage is less than 20%, the patient's condition will become worse.[18] In this study, after the diagnosis of viral pneumonia, the percentage of lymphocytes in all 10 patients was <20%, and the CD3/CD4 T cell/lymphocyte counts decreased in 7 patients. Compared with those without IPA, H1N1 patients with IPA had significantly lower absolute lymphocyte numbers and lymphocyte percentage, indicating their weakened immune function. After 7 to 14 days of antiviral therapy, the percentage of lymphocytes and CD3/CD4 T cell/lymphocyte returned to normal in 5 patients. The absolute lymphocyte numbers after treatment in H1N1 patients with IPA were significantly higher, suggesting that their immune function is improved after treatment.

Influenza viruses can cause cell-mediated airway epithelial destruction, which in turn contributes to the colonization of Aspergillus. Meanwhile, influenza-induced ARDS and hypoxemia may lead to impaired immune function.[19] In Europe, it has been reported that the incidence of influenza IPA was 14%.[9] Of the 47 cases of H1N1 in this study, 21.28% were diagnosed/clinically diagnosed as IPA, and the incidence was slightly higher than that in Europe. In 2018, the average rainfall in Kunming, China increased by 23% compared with the same period of history, and the average temperature was 0.5°C to 1.0°C higher than the same period of history. The high temperature and humid weather may cause the proliferation of Aspergillus, which may be related to the incidence of IPA following influenza A.

Influenza A (H1N1) virus pneumonia is an interstitial pneumonia.[20] Most patients with influenza and IPA have no characteristic imaging changes in the early stage.[21] It is generally believed that abnormalities in chest imaging may be observed at 5 to 7 days after the onset of influenza. Typical CT findings are nodular density with increased subpleural density and halo sign, crescent sign in the center of the lesion, ground-glass opacities, and aspergillus bulbs. However, traditional imaging features occur only in 5% of critically ill patients.[4] Liss et al[22] reported that the subpleural halo sign was a characteristic imaging change in the early stages of IPA. In the subjects of this study, 2 patients developed halo sign after IPA. Recently, there have also been studies using new imaging measures including PET-CT, etc, which are considered to be able to diagnose IPA and distinguish whether the infection is invasive or noninvasive.[23,24] However, for patients with early-stage influenza and IPA, because the imaging performance is not typical, imaging findings can only be highly recommended for diagnosis but cannot be used as the diagnostic standard.

The diagnosis of IPA requires histopathological examination or positive culture of Aspergillus from normal sterile sites, but pathogenic culture is limited by time and culture environment. In this study, only 1 of the 10 patients was tested for Aspergillus fumigatus in BALF culture, and 2 patients were tested for

Aspergillus by Metagenomics Next-Generation Sequencing. The above results indicate that the positive rate of Aspergillus culture is not high, which makes it difficult to obtain microbial and histopathological diagnosis in clinic. In recent years, clinical studies have found that positive BALF GM test has a high value for the diagnosis of early Aspergillus infection.[9,25] However, the cutoff point of GM OD has been controversial. In patients who were diagnosed with IPA by autopsy and biopsy, the sensitivity of BALF GM OD > 0.5 was 88%. In an intensive care unit observational study, the value of BALF GM was questioned because of specificity. Compared with patients with positive BALF cultures, the specificity of OD > 1.0 was 38%, and that of OD > 3.0 was 62%.[26] Studies have shown that the specificity of BALF GM is better when the OD value is 1.0, and the specificity of serum GM is also considered to be better.[9] In this group of patients, when GM OD>0.5, 9 patients had elevated BALF GM, and 6 patients had elevated serum GM. After 2 weeks of treatment with voriconazole, 5 cases of BALF GM decreased to normal, and serum GM decreased to normal. After 4 weeks of treatment, BALF GM of 4 patients returned to normal, and only 1 patient was still with increased BALF GM. Therefore, for patients with IPA who have difficulty obtaining pathogenic evidence in the early stage, BALF and serum GM tests can provide evidence for early diagnosis and evaluation of the patient's condition.

Studies have shown that the mortality rate of influenza-related IPA patients in the ICU is 45%,[4,7,9,27] and the mortality rate may be related to the disease severity, and the time of diagnosis and treatment. Although it is not certain that delayed antifungal therapy can lead to a serious prognosis,[28] a shortened initial treatment time can reduce IPA mortality. In this study, 10 patients were treated with empirical anti-Aspergillus at early stage, and the initial treatment time was 1 to 2 days after clinical diagnosis (median time 1 day). After treatment, the symptoms of the patients were alleviated significantly. It indicates that early empirical anti-Aspergillus treatment can benefit patients with influenza-related IPA.

This study has some limitations. First, this is a single-center study. The number of cases included in the study is small, and the sample size needs to be further increased. Second, all the selected patients were diagnosed with influenza A, but most patients had other underlying diseases. Therefore, we cannot exclude the possibility that patients may suffer from IPA due to the weakened immune function caused by the underlying disease. Third, the diagnosis of IPA requires pathological examination. In this study, Aspergillus was detected in only 2 patients pathologically, and the diagnosis of the remaining 8 cases depended on the GM test. At present, there is no clear diagnostic criteria for GM, and the criteria selected used in this study were relatively low (GM > 0.5). The possibility of false positives cannot be completely ruled out, which is one big limitation of this study. The GM standard positive rate needs to be further improved.

In conclusion, the data of clinical manifestations, laboratory examination results, chest CT, and treatments of patients with IPA following H1N1 infection were clearly summarized. Our findings may improve the early detection rate of IPA. All patients were discharged after empirical anti-aspergillus treatment. But due to the small sample size, it is necessary to further expand the sample size in the future to better summarize the clinical characteristics, thereby standardizing early diagnosis and treatment and hence reducing patient mortality and improving survival rate.

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