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ORIGINAL ARTICLE

Invasive pulmonary aspergillosis in patients with influenza infection: A retrospective study and review of the literature

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Abstract

Introduction: There has been a rapid increase in the number of influenza and invasive pulmonary aspergillosis (IPA) co-infection.

Objectives: To explore the risk factors and predictors of a poor prognosis in influenza and IPA co-infection.

Methods: We included patients with confirmed influenza during the 2017-2018 influenza season and cases of influenza and IPA co-infection in the literature.

Results: A total of 64 patients with influenza infection were admitted to ICU. Of these patients, 18 were co-infected with IPA. Others were assigned to the control group ($n = 46$). A total of 45 patients from the literature were added to the IPA group ($n = 63$). A multivariate logistic regression suggested that influenza patients who were given steroids after ICU admission, who had a white blood count (WBC) of more than $10*10^9$/L on ICU admission and whose CT findings manifested as multiple nodules and cavities might have a higher risk of developing IPA. Compared to survivors, non-survivors had higher sequential organ failure assessment (SOFA) scores (16 ± 4 points vs 8 ± 4 points, $P < 0.001$), lower $CD4^+$ T cell counts on ICU admission [315 (83-466) cells/μL vs 152 (50-220) cells/μL, $P = 0.031$] and more requirement extracorporeal membrane oxygenation (ECMO) support [13 (50%) vs 7 (18.9%), $P = 0.015$].

Conclusions: Influenza patients who are given steroids after ICU admission, who have WBCs of greater than $10*10^9$/L on ICU admission, and whose CT imaging shows multiple nodules and cavities might have a high risk of IPA. Higher SOFA scores, $CD4^+$ T cell counts lower than 200 cells/μL on ICU admission and more ECMO requirement might be predictors of a poor prognosis.

KEYWORDS

clinical presentations, influenza, invasive pulmonary aspergillosis (IPA), prognosis, risk factor

Abbreviations: AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; CHF, chronic heart failure; CRBSI, catheter-related bloodstream infection; CT, computed tomography; CTD, connective tissue disease; DM, diabetes mellitus; ECMO, extracorporeal membrane oxygenation; GGO, ground glass opacity; GM, galactomannan; IPA, invasive pulmonary aspergillosis; IPPV, invasive positive pressure ventilation; LRT, lower respiratory tract; MICU, medical intensive care unit; PCR, polymerase chain reaction; PCT, procalcitonin; SOFA, sequential organ failure assessment; VAP, ventilation associated pneumonia; WBC, white blood count.

Linna Huang and Nannan Zhang contributed equally to the article.

1 | INTRODUCTION

Co-infection as a common complication of influenza infection that has been well described, and it often contributes to increased morbidity and mortality.[1] Bacterial pathogens, often including Streptococcus and Staphylococcus aureus, have become considerable issues,[2] while co-infection with Aspergillus has typically been ignored. However, it might play a role in severe influenza cases.

Invasive Aspergillus infections are a well-known burden for immunocompromised patients. While Aspergillus is not traditionally thought of as a pathogen capable of invasive infection following a viral respiratory infection, since 2010, there has been a rapid increase in the amount of research investigating influenza and subsequent invasive Aspergillus co-infection.[3]

Influenza may represent a novel host risk factor for this invasive fungal infection. Meanwhile, the mortality rate among co-infected patients has been found to be approximately 50%-60%,[4] which is approximately 5 times that of hospitalized patients with influenza alone.

Invasive pulmonary aspergillosis (IPA) co-infections with influenza have been sporadically reported in case reports and small case series,[5-7] but no systemic research has yet been aimed at investigating the clinical characteristics of IPA co-infections with influenza, how to recognize it earlier and its poor prognostic factors.

Our study included patients with IPA complicating severe influenza infections who were admitted to the medical intensive care unit (MICU) of the China-Japan Friendship Hospital during the 2017-2018 influenza season. In addition, cases from a comprehensive review of the English literature were added to provide a better and more comprehensive view of the clinical features, potential risk factors and predictors of the prognosis of influenza combined with IPA.

2 | MATERIALS AND METHODS

2.1 | Study population and group divisions

2.1.1 | Our cases

We retrospectively included all confirmed influenza-infected patients with respiratory failure admitted to the MICU of the China-Japan Friendship Hospital from November 2017 to March 2018.

2.1.2 | Cases from the literature

In addition to our current cases, we searched the English language published literature using PubMed/Medline with the search terms 'influenza' and 'aspergillus' or 'aspergillosis' from January 2009 to March 2018.[1,5-18]

2.1.3 | Group division

We divided the patients into two groups. The case group included patients with confirmed influenza infection who subsequently became infected with proven and/or probable IPA, and the control group included patients with confirmed influenza infection who showed no evidence of Aspergillus infection while hospitalized.

Cases involving nonspecific viral infections or without confirmatory influenza testing, reports with insufficient patient information and with possible IPA or Aspergillus colonization were excluded.

2.2 | Diagnostic criteria

2.2.1 | Definition of influenza virus infection

All patients with influenza had testing performed using a nasopharyngeal swab and lower respiratory tract (LRT) specimens. Three methods were used for a laboratory diagnosis, namely a polymerase chain reaction or a respiratory viral culture along with serological testing.[3,19]

2.2.2 | Definition of IPA

Proven IPA is defined as microscopic evidence of dichotomous branching hyphae with a positive culture for Aspergillus spp through an endobronchial biopsy, irrespective of host factors or clinical features.[20] Probable IPA requires a host factor, clinical features and mycological evidence of aspergillosis. One of the three criteria[20-22] for an IPA diagnosis is host factors; however, these criteria were largely created for immunosuppressed hosts, and influenza-related aspergillosis may occur in previously normal hosts. Thus, the host factors criterion was not required in our study. The clinical signs and symptoms, radiological findings and mycological data should be met according to our newly proposed 'Modified Bulpa Criteria'[23] (Additional File 1).

2.3 | Data collection and analysis

Data were retrospectively collected from electronic medical records, including demographics, underlying diseases and the use of immunosuppressive agents and steroids before admission. Data on the use of steroids during the hospitalizations were recorded. Laboratory examinations included the influenza-type, white blood count (WBC) and lymphocyte and $CD4^+$ T lymphocyte counts. All respiratory culture data from the hospitalizations were evaluated. Radiological examinations, mainly chest computed tomography (CT), were reviewed. Complications during hospitalizations and needs for organ support were also recorded. Data regarding lengths

TABLE 1 Characteristics, radiological findings and mycological data of our patients

	Patients characteristics						Mycological data				
Case	Age	Sex	Underlying diseases	Time from influenza to IPA	Grade of IPA diagnosis	Radiological findings	Culture	BALF GM	Serum GM	Histo evidence	Outcome
1	87	F	Lung disease	21	Probable	Tree in bud	*Aspergillus fumigatus*	+	−	Not done	Improved
2	61	M	CTD	27	Probable	Nodules, mass, patchiness	None	+	−	Not done	Died
3	81	F	None	13	Probable	Tree in bud, GGO	None	−	+	Not done	Improved
4	51	M	None	6	Probable	Nodules, patchiness, large consolidation, cavities, reversed halo sign	None	+	−	Not done	Improved
5	49	M	None	22	Probable	Large consolidation, GGO	None	+	−	Not done	Died
6	46	M	DM	9	Probable	Nodules, patchiness, large consolidation, cavity	*Aspergillus fumigatus*	+	+	Not done	Improved
7	41	F	None	8	Probable	Nodules, patchiness, large consolidation, halo sign, cavity, GGO	*Aspergillus flavus*	+	−	Not done	Improved
8	51	M	DM	9	Probable	Patchiness, large consolidation, air-crescent sign, GGO	*Aspergillus fumigatus*	+	+	Not done	Improved
9	51	M	DM	18	Probable	Patchiness, large consolidation, cavity	None	+	+	Not done	Improved
10	63	M	CTD	16	Probable	Patchiness	*Aspergillus fumigatus*	−	+	Not done	Died
11	16	M	None	13	Probable	Nodules, patchiness, large consolidation, reversed halo sign, cavity	*Aspergillus fumigatus*	+	+	Not done	Died
12	46	F	DM	15	Proven	Nodules, patchiness, large consolidation, halo sign	*Aspergillus fumigatus*；*Aspergillus flavus*	+	+	Autopsy	Died
13	65	F	DM, lung diseases	7	Probable	Nodules, cavities	*Aspergillus fumigatus*	+	−	Not done	Improved
14	66	M	DM	23	Probable	Nodules, patchiness, cavities	None	+	+	Not done	Died
15	35	M	DM	10	Probable	Nodules, patchiness, large consolidation, cavities	*Aspergillus fumigatus*	+	−	Not done	Died
16	52	M	None	12	Probable	Nodules, patchiness, halo sign	*Aspergillus flavus*	+	+	Not done	Improved
17	76	F	DM, CHF, lung disease	2	Probable	Nodules, patchiness	None	+	−	Not done	Improved
18	75	M	Malignancy	5	Probable	Patchiness, large consolidation, cavity	None	+	−	Not done	Died

Abbreviations: CHF, chronic heart failure; CTD, connective tissue disease; DM, diabetes mellitus; GGO, ground glass opacity; GM, galactomannan; F, female; IPA, invasive pulmonary aspergillosis; M, male.

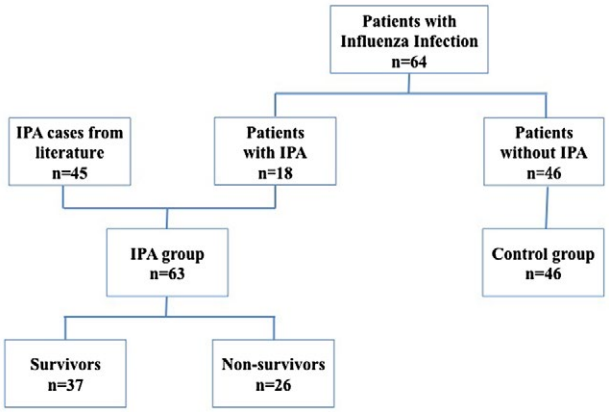

FIGURE 1 Flowchart. A flowchart illustrating the enrolment of patients in our study. From November 2017 to March 2018, 64 critically ill patients with confirmed influenza were admitted to the MICU at the China-Japan Friendship Hospital due to respiratory failure. Of these patients, 18 suffered from IPA, and the other 46 were designated as the control group. We added the IPA cases from the literature (45 cases) to our IPA cases (18 cases) to create the case group ($n = 63$). Out of the 63 patients included in the case group, 37 survived and 26 died

of MICU stays, total hospitalization days and mortality before discharge were collected as well. For those with aspergillosis, data on the number of days between the diagnoses of influenza and aspergillosis, the Aspergillus species from the LRT cultures, serum and/or BALF galactomannan levels and antifungal therapy information were recorded.

We first described the overview of this influenza pandemic (from November 2017 to March 2018) and IPA coinfection. We then added the IPA cases from the literature to our IPA cases to create the case group, while the other patients from our cases were placed in the control group. We compared the two groups in terms of demographics, underlying diseases, use of immunosuppressive agents and steroids before admission, steroid use during hospitalization and laboratory and radiological data to investigate the clinical presentation of influenza combined with IPA. Then, a logistic regression analysis was performed to explore the condition's risk factors. Finally, we compared survivors and non-survivors of IPA to determine predictors of a poor prognosis.

2.4 | Statistical analysis

All of the analyses were carried out using SPSS 16.0 software. Normally distributed continuous variables were expressed as $\bar{x} \pm SD$ and were compared using *t*-tests. Non-normally distributed continuous variables were expressed as medians and quartiles and were compared using Wilcoxon rank-sum tests. Categorical variables were compared using x^2 tests. A multivariate logistic regression analysis was used to identify independent risk factors and poor prognosis for influenza combined with IPA. Variables in bivariate analyses with *P* value less than 0.2, as well as the variables thought to be meaningful in practice even though with no statistical significance are all included in the model. Power analysis was performed by PASS 16 to verify whether the sample size was adequate to identify risk factors and poor prognosis in multivariate logistic regression analysis.

3 | RESULTS

3.1 | Overview of patients with influenza in the MICU in the 2017 to 2018 influenza season

From November 2017 to March 2018, 64 critically ill patients with confirmed influenza were admitted to the MICU at the China-Japan Friendship Hospital due to respiratory failure; the mortality rate for all of these influenza cases was 43.8%.

Among these patients, 18 suffered from IPA, giving an incidence of 28.1%. One patient was diagnosed with proven IPA using a trans-bronchial lung biopsy. The other 17 patients were diagnosed with probable IPA, and the mean time from the influenza diagnosis to the IPA diagnosis was 13 (±7) days. The average age of the patients was 52 (±18) years, 66.7% were male and the mean sequential organ failure assessment (SOFA) score was 8 (±4) points. Ten patients (55.6%) had underlying diseases, one (5.6%) had a history of immunosuppressant use and eight (44.4%) reported steroid use in the last month. Steroids were used after MICU admission in five patients. Invasive positive pressure ventilation (IPPV) was required for 16 patients (88.9%), and the average duration of its use was 14 (5-33) days. Rescue ventilation strategies and extracorporeal membrane oxygenation (ECMO) were needed for 10 (55.7%) and 4 (22.2%) patients, respectively; the average duration of ECMO use was 10 (6-23) days. The mean lengths of ICU and total hospital stays were 16 (6-35) days and 19 (10-36) days, respectively. The mortality rate was 44.4% (Table 1).

3.2 | Comparisons between IPA and non-IPA patients

We added the IPA cases from the literature (45 cases) to our IPA cases (18 cases) to create the IPA group ($n = 63$), while the other patients from our set of cases ($n = 46$) were designated as the control group (Figure 1).

3.2.1 | Patient characteristics and outcomes between the two groups

General information

The IPA and control groups did not differ significantly in terms of the number of cases of obesity, diabetes, underlying

lung disease, chronic heart failure, chronic kidney disease, steroid use and accumulated steroid dosage prior to ICU admission. Compared to the control group, the IPA group included more male patients (68.3% vs 47.8%, $P = 0.032$), was older (63 ± 13 years vs 57 ± 18, $P = 0.044$) and had more severe conditions (SOFA scores: 10 ± 3 points vs 7 ± 4 points, $P < 0.001$). The numbers of patients with a malignancy (23.8% vs 6.5%, $P = 0.016$), with immunosuppressant use in the past month (46% vs 19.6%, $P = 0.005$), and who were given steroids after ICU admission [234 (0-400) mg vs 89 (0-120) mg, $P = 0.036$] were higher in the IPA group than in the non-IPA group. Moreover, compared to the control group, the accumulated dose of steroids was much higher in the IPA group (Table 2).

Laboratory examinations and radiological findings

The IPA group had a higher WBC [$(10.2 \pm 4.6)*10^9$/L vs $(6.1 \pm 3.3)*10^9$/L, $P < 0.001$], and lower lymphocyte count [$(0.47 \pm 0.52)*10^9$/L vs $(0.67 \pm 0.46)*10^9$/L, $P = 0.035$] on ICU admission. $CD4^+$ T cell counts on ICU admission and types of influenza did not differ significantly between the two groups. Patchiness was seen more often in the CT imaging of non-IPA patients, while nodules (51.9% vs 21.1%, $P = 0.003$), and especially multiple nodules distributed along the airway bundles and cavities (30.8% vs 10.5%, $P = 0.013$) were seen more often in IPA patients. The incidences of masses, large consolidations and halo signs were similar between the two groups (Table 2).

Clinical outcomes

Despite there being no significant differences in mortality between the two groups, the lengths of ICU [25 (13-39) vs 12 (7-20), $P = 0.005$] and total hospital stays [28 (14-58) vs 18 (10-27), $P = 0.002$] was higher in the IPA group than in the control group (Table 2).

3.2.2 | Organ support needs and complications between the two groups

Compared to the control group, more patients in the IPA group needed rescue ventilation strategies (42.9% vs 21.7%, $P = 0.021$), renal replacement therapies (49.2% vs 28.3%, $P = 0.033$), and high-dose vasoactive drugs (68.3% vs 41.3%, $P = 0.005$). The numbers of patients needing IPPV and ECMO support did not differ significantly between the two groups. The duration of IPPV was significantly longer in the IPA group than in non-IPA group [22 (9-27) days vs 17 (7-18) days, $P = 0.047$], while the durations of renal replacement therapy and ECMO support did not differ significantly. For complications, patients in the IPA group were more prone to developing septic shock (with incidences of 66.7% vs 41.3%, $P < 0.001$). Other complications, such as the incidences of ventilator-associated pneumonia (VAP), catheter-related bloodstream infection (CRBSI), bacteremia, barotrauma and haemorrhage were similar between the two groups (Table 3).

3.3 | Subgroup analysis: comparison between survivors and non-survivors in the IPA group

Out of the 63 IPA patients included in this study, 26 died, giving a mortality rate of 41.3%. Compared to survivors, non-survivors were older (60 ± 15 years vs 67 ± 9 years, $P = 0.040$), had higher SOFA scores (16 ± 4 points vs 8 ± 4 points, $P < 0.001$) and had lower $CD4^+$ T cell counts on ICU admission [315 (83-466) cells/μL vs 152 (50-220) cells/μL, $P = 0.031$]. All of the non-survivors had influenza type A (100% vs 70.3%, $P = 0.002$), and more non-survivors required IPPV (96.2% vs 54.1%, $P < 0.001$), rescue ventilation strategies (57.7% vs 32.4%, $P = 0.046$) and ECMO support (50% vs 18.9%, $P = 0.015$) compared to survivors in the IPA group (Table 4).

3.4 | Multivariate logistic regression analysis for independent risk factors and the predictors of a poor prognosis for IPA in patients with influenza

To investigate the roles of potential confounding risk factors and predictors of poor prognosis of IPA, a multivariate logistic regression analysis was performed. We found that influenza patients who were given steroids after ICU admission, who had a WBC of more than $10*10^9$/L, and whose CT imaging showed multiple nodules and cavities might have a higher incidence of IPA. Higher SOFA scores, $CD4^+$ T cell counts lower than 200 cells/μL on ICU admission and more ECMO requirement might be predictors of a poor prognosis (Table 5).

The systemic steroid application was commonly regarded as the risk factor for influenza and IPA confections in studies before.[8] In this way, the 'Steroids after ICU admission' was identified as primary factor for power analysis. The power was 0.77, which might verify the sample size was adequate to identify risk factors.

4 | DISCUSSION

The biggest strength of our study is the large group of literature-based cases of IPA following influenza infection that was included. Additionally, this is the first study to systematically and comprehensively provide an overview of the clinical presentation of influenza combined with IPA and to explore the possible risk factors for its diagnosis and predictors of a poor prognosis.

TABLE 2 Patient characteristics and outcomes between the two groups

	IPA ($n = 63$)	Non-IPA ($n = 46$)	*P* Value
General information			
Age, years, mean ± SD	63 ± 13	57 ± 18	**0.044**[*]
Sex (men), number (%)	43 (68.3)	22 (47.8)	**0.032**[*]
BMI > 30 kg/m^2, number (%)	5 (7.9)	5 (10.9)	0.600
SOFA, mean ± SD	10 ± 3	7 ± 4	**0.001**[#]
Diabetes, number (%)	19 (30.2)	12 (26.1)	0.642
Underlying lung disease, number (%)	13 (20.6)	5 (10.9)	0.175
Chronic heart failure, number (%)	9 (14.3)	3(6.5)	0.201
Chronic kidney disease, number (%)	2 (3.2)	2 (4.3)	0.748
Malignancy, number (%)	15 (23.8)	3 (6.5)	**0.016**[*]
Immunosuppressant use in past month, number (%)	15 (23.8)	3 (6.5)	**0.016**[*]
Systemic steroid use			
Before ICU admission, number (%)	23 (36.5)	13 (28.3)	0.366
Accumulated dosage before ICU, mg, median (IQR)	242 (0-300)	238 (0-1092)	0.977
After ICU admission, number (%)	29 (46.0)	9 (19.6)	**0.005**[*]
Accumulated dosage after ICU, mg, median (IQR)	234 (40-400)	89 (0-120)	**0.036**[*]
Laboratory examination			
WBCs on ICU admission, *10^9/L, mean ± SD	10.2 ± 4.6	6.1 ± 3.3	**<0.001**[#]
Lymphocytes on ICU admission, *10^9/L, mean ± SD	0.47 ± 0.52	0.67 ± 0.46	**0.035**[*]
CD4$^+$ T cells on ICU admission, cell/μL, mean ± SD	239 (82-416)	320 (209-458)	0.373
Influenza A, number (%)	52 (82.5)	36 (78.3)	0.699
Influenza B, number (%)	11 (17.5)	11 (23.9)	0.384
Radiological findings			
Patchiness, number (%)	34 (65.4)	37 (97.4)	**<0.001**[#]
Nodules, number (%)	27 (51.9)	8 (21.1)	**0.003**[*]
Mass, number (%)	1 (1.9)	1 (2.6)	0.811
Large consolidations, number (%)	24 (46.2)	11 (28.9)	0.098
Halo sign, number (%)	5 (9.6)	0 (0.0)	0.051
Cavity/air-crescent sign, number (%)	16 (30.8)	4 (10.5)	**0.013**[*]
Outcomes			
Length of ICU stay, days (IQR)	25 (13-39)	12 (7-20)	**0.005**[*]
Length of hospitalization, days (IQR)	28 (14-58)	18 (10-27)	**0.002**[*]
Mortality, number (%)	26 (41.3)	20 (43.4)	0.818

Abbreviations: BMI, body mass index; ICU, intensive care unit; IQR, interquartile range; SD, standard deviation; SOFA, sequential organ failure assessment; WBC, white blood count.

The bold values indicates *P* values from 0.01 to 0.05.

Note. The steroid doses were converted to prednisone doses.

[*] $P < 0.05$;

[#] $P < 0.01$.

Our study revealed that the incidence of IPA co-infection in the influenza season examined here was 28.1%. A study by Nancy F. in 2016[3] showed that 5 (62.5%) of 8 ICU influenza cases were classified as an invasive disease, which was much higher than in our cases. A rising number of recent cases have revealed that influenza might represent a novel host risk factor for invasive fungal infection. The pathogenesis of IPA in the setting of influenza infection may occur as follows. The influenza virus may cause severe and diffuse damage to the respiratory mucosa and disrupt normal ciliary clearance,[24,25] allowing for fungal invasion. Influenza may also impair local phagocytosis by alveolar macrophages as well as reduce natural killer cell functionality and other immune responses.[26,27]

The mortality rate among our IPA cases was 44.4%, and this changed to 41.3% after the cases from the literature were added. The mortality rate in critically ill patients with

TABLE 3 Organ support needs and complications between the two groups

	IPA ($n = 63$)	Non-IPA ($n = 46$)	*P* Value
Organ support needs			
IPPV, number (%)	45 (71.4)	34 (73.9)	0.774
IPPV days, days, median (IQR)	22 (9-27)	17 (7-18)	**0.047**[*]
Rescue ventilation strategies,[a] number (%)	27 (42.9)	10 (21.7)	**0.021**[*]
ECMO, number (%)	20 (31.7)	11 (23.9)	0.371
ECMO days, days, median (IQR)	11 (5-19)	13 (10-26)	0.455
Renal replacement therapy, number (%)	31 (49.2)	13 (28.3)	**0.033**[*]
Renal replacement therapy days, days, median (IQR)	7 (5-10)	7 (3-13)	0.677
High-dose vasoactive drugs,[b] number (%)	43 (68.3)	19 (41.3)	**0.005**[*]
Complications during therapy			
VAP, number (%)	32 (50.8)	20 (43.5)	0.450
CRBSI, number (%)	1 (1.6)	1 (2.2)	0.822
Bacteremia, number (%)	1 (1.6)	4 (8.7)	0.080
Barotrauma, number (%)	7 (11.1)	7 (15.2)	0.527
Haemorrhage, number (%)	7 (11.1)	6 (13.0)	0.759
Septic shock, number (%)	42 (66.7)	19 (41.3)	**<0.001**[#]

Abbreviations: CRBSI, catheter-related bloodstream infection; ECMO, extracorporeal membrane oxygenation; IPPV, invasive positive pressure ventilation; IQR, interquartile range; VAP, ventilator-associated pneumonia.
The bold values indicates *P* values from 0.01 to 0.05.
[a]Rescue ventilation strategies included the recruitment manoeuvre (RM), prone position ventilation (PP) and high-frequency oscillatory ventilation (HFOV).
[b]High-dose vasoactive drugs refer to: noradrenaline >0.5 μg/kg/min or dopamine >20 μg/kg/min.
[*]$P < 0.05$;
[#]$P < 0.01$.

influenza-associated IPA was reported to be 59%-95%.[28] A lack of awareness, the interference of underlying influenza, and the limited use of serological diagnostic methods in some medical institutions impeded the prompt diagnosis of IPA co-infection, delayed therapies and thus led to a high mortality rate. With the high incidence and mortality rates observed, awareness should be raised regarding influenza as an important risk factor for IPA co-infection, and medical care providers should watch for IPA during the course of influenza.

A multivariate logistic regression analysis suggested that the influenza patients who were treated with steroids after ICU admission, those with WBCs of more than $10*10^9$/L on ICU admission, and those whose CT imaging manifested as multiple nodules and cavities might have a high risk of IPA co-infection. According to reports, steroids are used in the treatment of 18%-69% of patients with influenza-associated acute respiratory distress syndrome (ARDS).[29] The benefits of limited inflammation in lung parenchyma with steroid use were not clear; however, the adverse effects, especially the immunosuppressive effects, might enable the growth of Aspergillus spp and hinder the efficacy of antifungal therapy.[7] A study by Joost Wauters and his colleague[7] revealed that the use of steroid 7 days before ICU admission is an independent risk factor for fungal co-infection. Currently, it is well established that systemic corticosteroids increase the risk of IPA. In our study, the number of patients in the IPA group who received systemic steroids after ICU admission was 46%, which was much higher than in the non-IPA group, and the accumulated dose was up to 234 (40-400) mg, which was also higher than the level in the control group. Patients with influenza who are on corticosteroids before and/or after ICU admission should be carefully evaluated for possible Aspergillus co-infection; we also suggest that systemic steroids should be avoided in patients with influenza, even those with influenza-associated severe ARDS.

Patients with IPA in non-classic immunosuppressive hosts usually had high WBCs, which were distributed to the excessive inflammatory responses.[21] This could account for the findings of multiple organ failure, such as acute kidney injury (49.2% vs 48.3, $P = 0.033$), and septic shock (66.7% vs 41.3, $P < 0.001$) that were observed more often in IPA patients compared with the control group. A WBC $>10*10^9$/L on ICU admission was an independent factor for IPA co-infection, especially when the procalcitonin (PCT) level, which indicates a bacterial infection, was normal. A lower lymphocyte count on ICU admission [$(0.47 \pm 0.52)*10^9$/L vs $(0.67 \pm 0.46)*10^9$/L, $P = 0.035$], even if it is not an independent risk factor for IPA, should cause awareness of this

TABLE 4 Comparisons between survivors and non-survivors of IPA

	Survivors ($n = 37$)	Non-survivors ($n = 26$)	*P* Value
General information			
Age, years, mean ± SD	60 ± 15	67 ± 9	**0.040**[*]
Sex (men), number (%)	26 (70.3)	19 (73.1)	0.808
BMI > 30 kg/m^2, number (%)	2 (5.4)	3 (11.5)	0.375
SOFA, mean ± SD	8 ± 4	16 ± 4	**<0.001**[#]
Diabetes, number (%)	14 (37.8)	5 (19.2)	0.113
Underlying lung disease, number (%)	7 (18.9)	6 (23.1)	0.688
Chronic heart failure, number (%)	5 (13.5)	4 (15.4)	0.834
Chronic kidney disease, number (%)	2 (5.4)	0 (0.0)	0.228
Malignancy, number (%)	3 (8.1)	3 (11.5)	0.648
Immunosuppressant use in past 3 months, number (%)	10 (27.0)	5 (23.1)	0.474
Systemic steroid use			
Before ICU admission, number (%)	14 (37.8)	9 (34.6)	0.794
Accumulated dosage before ICU, mg, median (IQR)	391 (50-450)	892 (50-2365)	0.064
After ICU admission, number (%)	16 (43.2)	13 (50.0)	0.596
Accumulated dosage after ICU, mg, median (IQR)	509 (350-500)	574 (375-500)	0.520
Time from onset to IPA diagnosis, days, mean ± SD	9 (4-14)	10 (5-16)	0.518
Laboratory examinations			
Lymphocytes on ICU admission, *10^9/L, median (IQR)	0.54 (0.22-0.54)	0.38 (0.15-0.53)	0.271
CD4$^+$ T cells on ICU admission, cell/μL, median (IQR)	315 (83-466)	152 (50-220)	**0.031**[*]
Influenza A, number (%)	26 (70.3)	26 (100.0)	**0.002**[*]
Organ failure and support			
IPPV, number (%)	20 (54.1)	25 (96.2)	**<0.001**[#]
Rescue ventilation strategies, number (%)	12 (32.4)	15 (57.7)	**0.046**[*]
ECMO, number (%)	7 (18.9)	13 (50.0)	**0.015**[*]
Renal replacement therapy, number (%)	17(45.9)	14 (53.8)	0.537
High-dose vasoactive drugs, number (%)	17 (45.9)	16 (61.5)	0.222

Abbreviations: BMI, body mass index; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; IPPV, invasive positive pressure ventilation; IQR, interquartile range; SD, standard deviation; SOFA, sequential organ failure assessment; WBC, white blood cell.

The bold values indicates *P* values from 0.01 to 0.05.

Note. The steroid doses were converted to prednisone doses.

[*] $P < 0.05$;

[#] $P < 0.01$.

possibility. Lymphopenia could lead to an alteration in the Th1 and Th2 balance, which was inclined to develop IPA.[3]

Radiological features in critically ill IPA patients are usually nonspecific, especially in patients with influenza-associated ARDS or bacterial co-infection. Our study suggested that multiple nodules were independent risk factors pointing to IPA in influenza patients. Multiple nodules distributed along the airway were common in non-classic immunosuppressive hosts.[30] These lung injuries are mainly due to an excessive host response rather than the fungal invasion itself, which mostly invades the vasculature, inducing secondary infraction; histological examinations of non-classic immunosuppressive patients often show large foci of pneumonia and exudative bronchiolitis with bronchial and alveolar destruction. Thus, multiple nodules distributed along the airway were also relatively specific signs of IPA co-infection in influenza patients and should be recognized promptly.

Our study revealed that lower CD4$^+$ T cell counts might predict a poor prognosis for IPA, which could be explained as patients lower CD4$^+$ T cell counts might have weaker cellular immunity, making them much more vulnerable to Aspergillus.

Our study had some limitations. Its retrospective nature was the largest problem, along with some missing data might influence the expected statistical outcome in some degree. Although we included cases from after 2009, there was still heterogeneity among influenza strains, treatment strategies and geographic factors. Meanwhile, it is very difficult to suspect co-infections

TABLE 5 Multivariate logistic regression analysis for risk factors and predictors of prognosis for IPA in patients with influenza

	Wald	*P* value	Odds ratio	95% confidence interval	
				Inferior	Superior
Risk factor for IPA co-infection					
WBCs (>10*10^9/L) on ICU admission	3.749	0.024	1.226	1.028	1.463
Steroids after ICU admission	3.568	0.045	5.372	0.980	29.460
CT indicated multiple nodules	11.902	0.001	9.833	1.965	49.194
CT indicated cavities	6.197	0.013	9.013	1.596	50.890
Predictors of prognosis for IPA					
SOFA	6.974	0.008	19.883	2.154	177.361
ECMO needed	5.211	0.022	1.210	1.027	1.432
$CD4^+$ T cells (<200 cell/μL) on ICU admission	4.362	0.038	8.661	1.214	68.334

Abbreviations: CT, computed tomography; ICU, intensive care unit; SOFA, sequential organ failure assessment; WBC, white blood count.

and secondary infections in clinical practice; however, retrospective nature means data that might help identify features of co-infection was not consistently captured. Prospective, multicentre studies are desired in order to elucidate the risk factors, clinical presentations and predictors of outcomes.

5 | CONCLUSIONS

With its high incidence and mortality, there should be greater awareness of influenza as an important risk factor for IPA co-infection. Influenza patients who receive steroids after ICU admission, who have WBCs of greater than 10*10^9/L on ICU admission, and whose CT imaging shows multiple nodules and cavities might have a high risk of developing IPA. Additionally, poor prognoses may be predicted for patients with higher SOFA scores, $CD4^+$ T cell counts lower than 200 cells/μL on ICU admission and more ECMO requirement.

AVAILABILITY OF DATA AND MATERIALS

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

AUTHORS CONTRIBUTIONS

All authors made substantial contributions to the conception and design of the study or to the data acquisition, analysis or interpretation; reviewed and approved the final manuscript; and significantly contributed to this study.

Study design: Qingyuan Zhan

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ETHICS

This study was approved by the ethics committee of the China-Japan Friendship Hospital, Beijing, PR China and written informed consent was obtained from the patients or their next of kin.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

FILE S1. Modified Bulpa Criteria. Probable IPA in our study was diagnosed using the 'Modified Bulpa Criteria'.[24]

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