Factors associated with fatality due to avian influenza A(H7N9) infection in China

Running title: Fatality due to avian influenza A(H7N9)

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Summary of the article's main point: Characterization of 350 hospitalized avian influenza A(H7N9) infected patients in China shows that age >65, secondary bacterial infections, and initiation of neuraminidase inhibitors therapy after 5 days from symptom onset were associated with increased risk of death.

Abstract

Background: The high case fatality rate of influenza A H7N9 infected patients has been a major clinical concern.

Methods: To identify the common causes of death due to H7N9 as well as identify risk factors associated with the high inpatient mortality, we retrospectively collected clinical treatment information from 350 hospitalized human cases of H7N9 virus in mainland China during 2013-2017, of which 109 (31.1%) had died, and systematically analysed the patient's clinical characteristics and risk factors for death.

Results: The median age of infection was 57 years, whereas the median age of mortality was 61 years, significantly older than those survived. In contrast to previous studies, we found nosocomial infections, comprising *Acinetobacter baumannii* and *Klebsiella* most commonly associated with secondary bacterial infections, which was likely due to the high utilization of supportive therapies, including mechanical ventilation (52.6%), ECMO (14%), CRRT (19.1%), and artificial liver therapy (9.7%). Age, time from illness onset to antiviral therapy initiation and secondary bacterial infection were independent risk factors for death. Age >65, secondary bacterial infections, and initiation of neuraminidase inhibitors therapy after 5 days from symptom onset were associated with increased risk of death.

Conclusions: Fatality among H7N9 virus infected patients occurred rapidly after hospital admission, especially among older patients, and was followed by severe hypoxemia and multisystem organ failure. Our results show that early neuraminidase-inhibitor therapy and reduction of secondary bacterial infections can help reduce mortality.

Key words: Influenza; H7N9; zoonotic infection; Risk factors

In the spring of 2013, a novel avian influenza A H7N9 virus was discovered in the Yangtze River Delta region of China [1], with patients presenting with rapid progression to acute respiratory distress syndrome (ARDS), septic shock, and even multiple organ failure, with high mortality [2-4]. Up to now, six annual waves of H7N9 virus epidemics have occurred in China, with 1220 known human infections, resulting in 494 deaths and a mortality rate of 40% [5]. Global health and safety is continued to be threatened by H7N9 virus, with the most recent case reported on April 4th, 2019 from Inner Mongolia, China [6].

The mortality rates due to H7N9 among the first five waves were 34%, 43%, 47%, 41% and 38%, respectively [5,7], showing that the mortality rate remained at a high level with no significant decline, despite continued virus evolution, indicating that a good treatment towards H7N9 infection was needed urgently. Thus, there is a great necessity to identify strategies for effective clinical treatment. Here we systematically analyzed the clinical features, treatment and prognosis of 350 confirmed cases of H7N9 virus infection during 2013–2017, evaluated the risk factors affecting the mortality rate, and report the high-risk factors directly related to mortality.

Materials and methods

Study design

In this study, we retrospectively collected information about 350 patients, who were clinically confirmed of H7N9 virus infection and hospitalized from different areas of China, including Zhejiang (186), Guangdong (61), Shanghai (30), Jiangsu (26), Hunan (22), Fujian (11), Anhui (3), Shandong (3), Henan (3), Guizhou (2), Beijing (2) and Hebei (1). The medical records of patients from these regions were sent to our data-collection center in Hangzhou, Zhejiang, where a team of physicians who had been taking care of patients with H7N9 virus infection reviewed the data. This study conformed to the ethical guidelines of the 2013 Declaration of Helsinki and was approved by the Institutional Review Board of the First Affiliated Hospital of Zhejiang University, Hangzhou. Further, the collection of the data from the H7N9 virus–infected patients was approved by The Chinese National Health and Family Planning Commission.

Data collection

The clinical data included demography, medical comorbidities, date of symptom onset, symptoms and signs, timing of antiviral therapy, progression and resolution of clinical illness. Medical comorbidities documented included diabetes mellitus, heart disease, chronic lung disease, renal failure, liver disease, human immunodeficiency virus infection, cancer, and receipt of immunosuppressive therapy, including corticosteroids. We considered that the symptoms started when any of fever, cough, chills, dizziness, headache, and fatigue appeared. Moderate-to-severe acute respiratory distress syndrome (ARDS) was diagnosed by definition of ARDS Berlin [8], severe hypoxemia ($PaO_2/FiO_2 \leq 200$ mmHg with PEEP ≥ 5 cmH_2O), in addition to bilateral opacities on chest X-ray that could not be fully explained by cardiac failure or fluid overload.

Laboratory confirmation

After admission, respiratory specimens (nasopharyngeal swabs, sputum, or endotracheal aspirates) were collected daily to determine the amount of H7N9 viral RNA by PCR analysis, as previously described [4]. Briefly, we used Taqman real time RT-PCR under standard thermo cycling conditions to detect the maxtrix (M), H7 haemaglutinin (HA) , and N9 neuraminidase (NA) genes. The detection limit of the M, H7, and N9 RT-PCR assays was approximately 100 copies of RNA per mL. Specimens with Ct values ≤38.0 were considered positive, specimens with Ct >38.0 were repeated, specimens with repeated results of Ct values <38 were considered positive, and specimens with Ct >38.0 and undetectable Ct values after repeated tests were considered negative.

Secondary infection was defined as recurrence of symptoms and signs of infection along with positive cultures of bacterial/fungal from lower respiratory tract specimens and/or blood after 48h admission.

Statistical analysis

For most variables, descriptive statistics, such as the mean standard deviation (SD; for data with normal distribution), median with interquartile range (IQR; for data with skewed distribution), and proportion (%), were calculated. The t-test, analysis of variance, Mann-Whitney U tests, Kruskal-Wallis tests were used for continuous variables. The χ^2 tests and Fisher exact test were used for categorical variables. The Kaplan-Meier curves were used to analyze survival, and logistic regression was used for multivariable analysis. Statistical analyses were performed using SPSS software, version 16.0 (SPSS). In all analyses, a *P* value <0.05 was considered significant. All probabilities were 2-tailed.

Results

Patient characteristics

Of 350 hospitalized H7N9 virus infected patients during 2013–2017, 109 (31.1%) had died. The demographic and clinical characteristics of H7N9 virus infected patients are shown in Table 1. The median age was 57 years old (IQR 46-67), and the ages of 28.6% of the patients were >65 years old. The median age of patients in the death group was significantly older than that of the survival group [61 (55-71.5) vs. 55 (42-65), *P*<0.001]. There were 234 male cases (66.9%) and no statistical difference in gender existed between the two groups. 209 cases (59.7%) had at least one underlying disease Hypertension (n=149) and diabetes mellitus (n=67) was the largest underlying medical conditions among H7N9 patients, both of the conditions were found to be significantly higher in the death group than in the survival group (50.5% vs. 39%, *P*=0.045; 25.7% vs. 16.2%, *P*=0.036, respectively), whereas 34 or fewer patients had either of cardiac, lung, kidney, liver, and tumor or were pregnant or immunosuppressed, with no statistical difference between the death and survived groups.

Clinical Features and Laboratory Abnormalities

Fever (97.1%), cough (90.9%), and expectoration (71.4%) were the most common clinical manifestations. In addition, 15.7% of patients developed gastrointestinal symptoms, however there was no significant difference between the survival group and the death group. Among laboratory indicators at admission, the oxygenation index (*P*<0.001), lymphocyte count (*P*=0.010), and platelet count (*P*=0.024) of fatalities were significantly lower than those survived, while K^+ (*P*=0.001), Aspartate aminotransferase (*P*<0.001), lactate dehydrogenase (*P*<0.001), creatine kinase (*P*=0.008), creatinine (*P*=0.007), calcitonin (*P*<0.001), and C-reactive protein (*P*=0.001) were significantly higher than those of the surviving cases. Inflammation had involved both lungs in 243 cases (69.4%), however there was no significant difference in lung imaging between the two groups (Table 1).

Treatment and Clinical Outcomes

All 350 patients received supportive treatments, including mechanical ventilation (52.6%), ECMO (14%), CRRT (19.1%), and artificial liver therapy (9.7%), the proportion of death cases receiving these treatments was significantly higher than that of survival cases (Table 2). While a majority of patients (325; 92.9%) were treated with antibiotics, 129 (36.9%) received intravenous infusion of gamma globulin. The median time from symptom onset to antibiotic use was 6 days (IQR 4-8), and the median duration of antibiotic therapy was 17 days (9-27.5). The proportion of patients treated with more than three antibiotics in the fatal group was significantly higher than in those that survived (*P*<0.001). Of 266 patients that used antibiotics, 181 used antibiotics before hospitalization and 74 (40.9%) developed secondary infection. A similar proportion of patients who received antibiotics after hospitalization (37.6%) developed secondary infection (*P*=0.834).

The proportion of corticosteroid usage was 79.1% among all patients, significantly higher in the death group than in the survived group (93.6% vs. 72.6%, *P*<0.001), and the largest dosage of corticosteroid in death group was significantly higher than that of the surviving group (80 [40-140] vs. 80 [40-80], P=0.003). All patients in the survived group received NAI antiviral treatment, while the NAI antiviral treatment rate in the death group was 97.2%, and the difference was statistically significant (*P*=0.030). In addition, 40.7% of patients in the survival group received oseltamivir-peramivir treatment, with statistically significant difference compared to the death group (*P*=0.004).

In this study, the ICU admission rate of the patients was 71.4%, the median time from symptom onset to ICU was 7 days (IQR 5-10), and the median time to stay in ICU was 15 days (IQR 7-35.8). Compared with the survival group, the death group had a higher rate of ICU admission (92.7% vs. 61.8%, P< 0.001). The proportion of concurrent shock and secondary infection in the death group was 72.5% and 54.1%, respectively, significantly higher than those of the survival group (Table 2). Among the 109 death cases, refractory hypoxemia was the most common cause of death, accounting for a total of 59 cases, followed by 20 cases of MODS, 18 cases of septic shock, 5 cases of acute heart failure, 1 case of arrhythmia, and 1 case of pulmonary embolism (Figure 1). The causes of the remaining 3 deaths were unknown.

Secondary bacterial infections

141 (40.3%) patients were co-infected with bacteria, among them 47 (33.3%) were positive for blood culture, 137 (97.2%) were positive for alfalfa and/or alveolar lavage, and 172 (7.1%) were positive for pleural fluid culture. The most common pathogen in blood culture was *Acinetobacter baumannii*, accounting for 19 cases (40.4%). Other common pathogens were *Klebsiella pneumoniae* in 11 cases (23.4%), *Enterococcus* in 9 cases (19.2%) and *Burkholderia cepacia* in 7 cases (14.9%). *Acinetobacter baumannii* is also the most common pathogen in sputum and/or BALF (Bronchoalveolar lavage fluid) culture, accounting for a total of 89 cases (65%), followed by 43 cases (24.8%) of *Klebsiella pneumoniae*, 24 cases (17.5%) of *Burkholderia cepacia*, 17 cases (12.4%) of *Aspergillus*, 15 cases (11%) of *Pseudomonas aeruginosa* and 10 cases (7.3%) of *Stenotrophomonas maltophilia*, etc. In the pleural effusion, 4 cases (40%) of *Klebsiella pneumoniae*, 2 cases (20%) of *Acinetobacter baumannii* and 1 case (10%) of *Candida* were cultured (Table 3).

Risk Factors for H7N9-Related Hospitalization

We conducted multivariate logistic regression analysis to identify risk factors associated with hospitalization and mortality (Table 4). Age, time from illness onset to antiviral therapy initiation, and secondary infection were identified as predictors (independent factors) of fatality, whereas gender, currently smoking, hypertension, heart diseases, diabetes, and COPD were not independent factors.

Using a Kaplan-Meier survival analysis we found that the delayed mortality (90 days post symptom onset) of H7N9-infected patients whose age was ≤65 years was significantly lower than those aged >65 years. Delayed fatality was significantly greater among those with secondary bacterial infections ($P<0.05$; Figure 2B). Furthermore, we found that mortality was significantly lower in patients treated with NAI <5 days from illness onset ($P<0.05$; Figure 2C), however the underlying disease had no significant effect ($P>0.05$; Figure 2D).

Discussion

In this study, we retrospectively analyzed the clinical characteristics and treatment of 350 H7N9 infected patients in China, assessed their risk factors for death, and identified features important in the prevention and treatment of patients with severe respiratory failure. The median age of patients hospitalized with H7N9 was 57 years old, whereby 28.6% patients were over 65 years. These demographics were consistent with 1220 laboratory-confirmed human infections across China [5]. In contrast, the age distribution of H5N1 hospitalizations was significantly lower with a median age of 19 years (range, 0.25 to 86) – derived from a systematic review of H5N1 case data from 1997–2015 [9]. The key difference may be due to live-poultry markets being the main source of H7N9 infection, where the elderly are more likely to be exposed to the virus [10]. In addition, our results showed that age was an important risk factor for death in H7N9. Since, elderly are at increased risk of coexisting illnesses, as well as produce weak immune response, they are more susceptible to severe forms of disease than younger persons [1, 11, 12].

Fever and cough were the most common symptoms of H7N9 hospitalizations, similar to previous reports in H7N9 [2], however there was no significant differences in clinical symptoms between the H7N9 survivors and fatalities, consistent with H7N9 data from Guangdong [11]. In contrast, there was significantly higher symptoms, including fever, cough and vomiting among H5N1 fatalities than survivors in Thailand during 2004–2006 [13].

Previous studies reported that underlying disease conditions was one of the death risk factors in H7N9 infected patients [12, 14], however we found no statistical differences between patients with and without an underlying disease. A high proportion of H7N9 patients had hypertension and diabetes - both of which were significantly higher among the fatalities (Table 1), potentially identifying important risk factors in the prognosis of H7N9. Our study was underpowered to examine the relationship of other underlying medical conditions to H7N9 fatalities, as there were 35 or fewer patients that had any of cardiac, lung, kidney, liver, and tumor or were pregnant or immunosuppressed.

Despite substantial differences between studies, decrease of WBC, lymphocytes and PLT as well as the increase of AST, CK and LDH, were similar to those reported among H1N1pdm09 and H5N1 infected patients [13,15]. While clinical studies on H5N1 found that the degree of lymphopenia and thrombocytopenia were directly correlated to the disease prognosis [16, 17], our research showed that lymphocyte and PLT counts in death group were significantly lower than survivor group, while the levels of AST, CK and LDH were significantly higher than survivor group. A closer attention to these indicators is warranted during clinical treatment.

We have shown that the fatality rate among hospitalized H7N9 infected patients was 31.1%, which was lower than the fatality rate in H5N1 infected patients reported in Vietnam and Thailand ranging from 67 to 80% [16,17], but was much higher than pandemic H1N1 patients in 2009 [18]. We further analyzed the cause of death in H7N9 infected patients. Similar to Gao et al [2], 72.5% of fatal cases were associated with refractory hypoxemia or MODS. Upon H7N9 infection, the capillary endothelial cells and alveolar epithelial cells are damaged and alveolar membrane permeability is increased, which further leads to pulmonary interstitial and alveolar edema, lung surfactant decrease, small airway closure and alveolar atelectasis [19,20]; these changes in pathology and alveolar morphology can result in severe ventilation-perfusion imbalance, pulmonary shunt and dispersion disorders, and further causing refractory hypoxemia. Some patients may develop MODS, even death.

Corticosteroid treatment has been controversial in patients with severe pneumonia caused by influenza. On one hand, administration of corticosteroids during critical illness, including severe influenza, may attenuate this state of adrenal insufficiency and help to maintain homeostasis, and control dysregulation of the immune system [21]. On the other hand, the use of corticosteroid treatment during influenza virus infection can significantly prolong the virus's survival time in the body [22]. Clinical studies in H1N1 have shown that corticosteroid treatment fails to benefit patients with severe influenza pneumonia, and even increases the risk of death in H1N1 patients [23-25]. In the clinical treatment of H7N9, although neither the World Health Organization nor the National Health and Family Planning Commission of China recommend the use of corticosteroid treatment, studies show that most H7N9 patients are treated with corticosteroid treatment. Cao et al discovered that low dosage (25–150-mg/day methylprednisolone or its equivalent) of corticosteroid had no effect on the duration of H7N9 virus, whereas high dosage (>150 mg/day methylprednisolone or its equivalent) of corticosteroid could significantly increase the duration of H7N9 virus in the body [26]. In this study, we found that 79.1% of the patients were treated with corticosteroid, and the proportion of corticosteroid use in the death group was significantly higher than that in the survival group, and the maximum dose of corticosteroid used in the death group was higher than that in the survival group. There are a number of reasons for this, but the main one is that the death group is more severe. This interferes with our evaluation of the efficacy of corticosteroid treatment. Whether corticosteroid are effective in treating H7N9 patients remains to be studied.

The guideline for diagnosis and treatment of H7N9 issued by the World Health Organization and the National Health and Family Planning Commission of China recommends that NAI antiviral therapy to be administered at the early stage of H7N9. In our previous study, we confirmed that early administration of neuraminidase inhibitor can significantly shorten the duration of H7N9 infection and improve the prognosis of patients [27]. Here, we found that the risk of death was 1.590 times higher for treatment post 5 days of symptom onset, further confirming that the early use of neuraminidase inhibitor can significantly reduce the risk of death. Interestingly, it has been reported that the combination of Oseltamivir and peramivir does not improve efficacy in influenza virus infection [28], but in this study we found that the proportion of Oseltamivir and peramivir in the survived group was significantly higher. However, whether a combination of Oseltamivir and peramivir is better than a single antiviral treatment requires further and specific studies to clarify.

Secondary bacterial infection following influenza virus infection has been a key cause of severe illness and death. Influenza virus can directly destroy airway epithelial cells, induce apoptosis of epithelial cells, expose epithelial basement membrane, and increase the susceptibility to bacterial infection [29, 30]. Furthermore, during early stages of infection, there is an increased secretion of pro-inflammatory factors in the lung that lead to a large number of inflammatory cells in the lung causing alveolar epithelial cell injury and pulmonary edema, providing an invasive environment for secondary bacterial infection [29, 30]. Martin et al. found that the risk of death in patients with secondary bacterial infection after influenza A (H1N1) virus infection was twice as high as that in patients without secondary bacterial infection, and it was an important independent risk factor for severe disease and death [31]. We found that the risk of death in patients with A H7N9 virus infection who had secondary bacterial infections was 1.686 times higher than that in patients without secondary bacterial infections. However, while *Streptococcus pneumoniae* and *Staphylococcus aureus* have been sho3s as the he most common secondary bacterial infections [31, 32], we found *Acinetobacter baumannii* and *Klebsiella pneumoniae* most commonly. This is likely due to the higher proportion of severe patients with H7N9 were treated with mechanical ventilation and ECMO, increasing the risk of secondary infection in the hospital during the interventional treatment. Notably, *Acinetobacter baumannii* and *Klebsiella pneumoniae* are the main cause of nosocomial infections in China.

Several study limitations should be noted when interpreting the results. First, although this study included cases from twelve provinces in China, comprising more than 20 large hospitals, a unified therapeutic regimen was not followed, therefore the patients were not guaranteed to receive the same solution in addition, the level of treatment, care, medical treatment equipment is different in different hospital, these are likely to cause death factor analysis bias. This was despite the issuance of guideline for diagnosis and treatment of H7N9 at the beginning of the outbreak by the National Health and Family Planning Commission of China. Second, this study is a retrospective analysis, therefore, the possibility of recall bias cannot be completely ruled out. Third, the admission stage of all patients is not uniform, not all patients were included in the study from the beginning of the disease, some patients may be transferred to the superior hospital for treatment after the treatment of primary medical institutions is ineffective, which may also have an impact on analysis.

In conclusion, A H7N9 virus can cause high mortality induced by hypoxemia and multiple organ failure. Lung rescue therapies included mechanical ventilation and ECMO are both required. Some of the clinical laboratory indicators at admission were associated with disease progression. We analyzed the death factors of patients with H7N9 avian influenza and found that age, time from illness onset to antiviral therapy initiation and secondary infection were the main risk factors for the death of patients. Therefore, it is recommended to use antiviral drugs as early as possible and pay attention to reduce secondary bacterial infections during treatment.

Notes

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Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Table 1. Clinical characteristics of 350 patients with avian influenza A(H7N9) infection

Characteristic	Total (n=350)	Survived (n=241)	Death (n=109)	*P* value
Demographics, n (%)				
Age, y, median (IQR)	57 (46-67)	55 (42-65)	61 (55-71.5)	**<0.001**
>65y	100 (28.6)	59 (24.5)	41 (37.6)	**0.012**
Male sex	234 (66.9)	164 (68)	70 (64.2)	0.481
Current smokers	77 (22)	51 (21.2)	26 (23.9)	0.600
Underlying conditions, n (%)				
Any	209 (59.7)	139 (57.7)	70 (64.2)	0.248
Hypertension	149 (42.6)	94 (39)	55 (50.5)	**0.045**
Diabetes mellitus	67 (19.1)	39 (16.2)	28 (25.7)	**0.036**
Cardiac disease [b]	34 (9.7)	21 (8.7)	13 (11.9)	0.347
Chronic lung disease [a]	25 (7.1)	14 (5.8)	11 (10.1)	0.150
Tumor	14 (4)	10 (4.1)	4 (3.7)	1.000
Chronic kidney disease	13 (3.7)	7 (2.9)	6 (5.5)	0.380
Chronic liver disease	12 (3.4)	9 (3.7)	3 (2.8)	0.880
Immunosuppression [c]	6 (1.7)	6 (2.5)	0 (0)	0.183
Pregnancy	6 (1.7)	3 (1.2)	3 (2.8)	0.314
Presenting symptoms, n (%)				
Fever	340 (97.1)	234 (97.1)	106 (97.2)	1.000
Cough	318 (90.9)	221 (91.7)	97 (89)	0.415
Sputum	250 (71.4)	177 (73.4)	73 (67)	0.215
Weakness	134 (38.3)	98 (40.7)	36 (33)	0.174
Muscle soreness	81 (23.1)	62 (25.7)	19 (17.4)	0.088
Hemoptysis	53 (15.1)	38 (15.8)	15 (13.8)	0.628
Gastrointestinal symptom [d]	55 (15.7)	39 (16.2)	16 (14.7)	0.653

Initial laboratory findings, median (IQR)				
PaO_2, mmHg	67 (55-81)	69.8 (58-85)	58.1 (59.3-134.7)	**<0.001**
PaO_2/FiO_2	128.3 (80-199.2)	149.22 (92.6-231.6)	86.7 (59.25-134.2)	**<0.001**
Leukocyte count, 10^9/L	4.6 (3-7)	4.4 (3-6.9)	5 (3.1-7.8)	0.269
Lymphocyte count, 10^9/L	0.5 (0.3-0.7)	0.5 (0.4-0.7)	0.4 (0.3-0.6)	**0.010**
Hemoglobin, g/L	129 (115-143)	130 (117-142)	128 (111.3-143)	0.487
Platelet count, 10^9/L	123.5 (90.3-161.5)	128 (94-170.5)	114.5 (78.3-155.8)	**0.024**
K^+, mmol/L	3.8 (3.5-4.2)	3.8 (3.4-4.1)	3.96 (3.6-4.5)	**0.001**
Na^+, mmol/L	137 (133-140)	137 (133-140)	138 (134.8-142)	0.076
Aspartate aminotransferase, UI/L	66 (40-119)	61 (37-100)	86.7 (49-149)	**<0.001**
Lactate dehydrogenase, UI/L	570 (399.5-831.5)	511.5 (365-722.5)	684 (493-963)	**<0.001**
Creatine Kinase, UI/L	226 (92-611)	200 (85-547.9)	341.5 (127.3-802)	**0.008**
Creatinine, μmol/L	70 (55.2-89.2)	67.8 (55-84.2)	79 (58.1-115)	**0.007**
Procalcitonin, ng/mL	0.4 (0.2-1.9)	0.3 (0.1-0.8)	1.3 (0.4-6.1)	**<0.001**
C-reactive protein, mg/L	81.7 (40.6-129)	73.9 (33.1-121.3)	97.2 (61.4-144.9)	**0.001**
Initial radiology findings, n (%)				
≥ 2 quadrants with infiltrate	243 (69.4)	169 (70.1)	74 (67.9)	0.674

Categorical variables were presented as number (%), and continuous variables as median (interquartile range).

[a] Chronic lung disease included chronic obstructive pulmonary disease and interstitial lung disease.

[b] Cardiac disease included coronary heart disease, valvular heart disease and congestive heart disease.

[c] Immunosuppression defined as the receipt of chemotherapy, radiotherapy or corticosteroid therapy within one month before illness onset.

[d] Gastrointestinal symptoms were any of the follows: nausea, vomiting, abdominal pain or diarrhea.

Table 2. Treatments and clinical outcomes of 350 patients with avian influenza A(H7N9)

Variable	Total (n=350)	Survived (n=241)	Death (n=109)	*P* value
Treatments, n (%)				
Mechanical ventilation	184 (52.6)	81 (33.6)	103 (94.5)	**<0.001**
Time from illness onset to MV start, d, median (IQR)	7 (5-9)	7 (6-10)	7 (5-9)	0.598
Duration of MV treatment, d, median (IQR)	17.1 (7-36.1)	20 (11-47)	13 (5.2-27)	**0.004**
Extracorporeal membrane oxygenation	49 (14)	22 (9.1)	27 (24.8)	**<0.001**
Continuous renal replacement therapy	67 (19.1)	25 (10.4)	42 (38.5)	**<0.001**
Artificial liver support	34 (9.7)	17 (7.1)	17 (15.6)	**0.012**
IV immunoglobulin	129 (36.9)	86 (35.7)	43 (39.4)	0.477
Antibiotic treatment	325 (92.9)	216 (89.6)	109 (100)	**0.001**
Time from illness onset to antibiotic start, d, median (IQR)	6 (4-8)	6.09 (4-9)	6 (2-7)	**0.015**
Duration of antibiotic treatment, d, median (IQR)	17 (9-27.5)	18 (10-28)	15 (5-27)	0.115
Types of antibiotics used, No., median (IQR)	2 (1-4)	2 (1-4)	3 (2-5)	**<0.001**
Antibiotic ≥ 3 classes	149 (42.6)	96 (39.8)	53 (48.6)	0.124
Corticosteroid treatment	277 (79.1)	175 (72.6)	102 (93.6)	**<0.001**
Time from illness onset to corticosteroid start, d, median (IQR)	7 (5-10)	7 (5-10)	7 (5-10)	0.996
Duration of corticosteroid treatment, d, median (IQR)	7 (4-12)	8 (4-12)	6 (3-12.5)	0.134
Initial dosage (equivalent methyiprednisolone), mg/d, median (IQR)	60 (40-80)	60 (40-80)	80 (40-120)	0.104
Maximum dosage (equivalent methyiprednisolone), mg/d, median (IQR)	80 (40-115)	80 (40-80)	80 (40-140)	**0.003**
NAI treatment	347 (99.1)	241 (100)	106 (97.2)	**0.030**
Time from illness onset to NAI start, d, median (IQR)	6 (4-8)	6 (4-8)	6 (5-8)	0.256
Oseltamivir-peramivir combination therapy	125 (35.7)	98 (40.7)	27 (24.8)	**0.004**
Clinical outcomes, n (%)				
Shock	121 (34.6)	42 (17.4)	79 (72.5)	**<0.001**
Secondary infection	141 (40.3)	82 (34)	59 (54.1)	**<0.001**
Time from hospitalization to Secondary infection start*				
1 week	80 (22.9)	41 (17)	39 (35.8)	**<0.001**

2 weeks	35 (10)	26 (10.8)	9 (8.3)	0.465
3 weeks	6 (1.7)	4 (1.7)	2 (1.8)	1.000
4 weeks	1 (0.3)	1 (0.4)	0	1.000
More than 4 weeks	3 (0.9)	3 (1.2)	0	0.555
ICU admission	250 (71.4)	149 (61.8)	101 (92.7)	**<0.001**
Time from illness onset to ICU admission, d, median (IQR)	7 (5-10)	7 (5-10)	7 (5-10)	0.800
ICU length of stay, d, median (IQR)	15 (7-32.8)	15 (9-36.8)	14.5 (4-27)	0.093
Length of hospital stay, d, median (IQR)	18 (10.6-30.5)	18 (12-31)	15 (5-28.5)	**0.002**

* 16 strains without culture time recorded.

Categorical variables were presented as number (%), and continuous variables as median (interquartile range). *MV* Mechanical ventilation, *NAI* neuraminidase inhibitor, *ICU* intensive care unit.

Table 3. Secondary bacterial infections in the study population

Pathogen, n (%)	Number of patients (n =141)	Blood culture (n =47)	Sputum/BALF[a] culture (n =137)	Pleural effusion culture (n =10)
A. baumannii	91(64.5)	19(40.4)	89(65)	2(20)
K. pneumoniae	37(26.2)	11(23.4)	34(24.8)	4(40)
B. cepacia	27(19.1)	7(14. 9)	24(17.5)	0
Aspergillus spp.	17(12.1)	0	17(12.4)	0
P. aeruginosa	15(10.6)	1(2.1)	15(11)	0
Enterococcus spp.	13(9.2)	9(19.2)	4(2.9)	0
S. aureus	11(7.8)	3(6.4)	9 (6.6)	0
S. maltophilia	10(7.1)	0	10(7.3)	0
E. cloacae	5(3.5)	1(2.1)	4(2.9)	0
E. coli	5(3.5)	0	5(3.7)	0
R. mannitolilytica	5(3.5)	0	5(3.7)	0
H. influenza	2(1.4)	0	2(1.5)	0
Candida	5(3.5)	5(10.6)	- [b]	1(10)
Others	19(13.5)	4(8.5) [c]	15(12) [d]	0

[a] BALF Bronchoalveolar lavage fluid.

[b] *Candida* is considered a colonizer of the airway.

[c] Including *Streptococcus uberis* (n=1), *Burkholderia pickettii* (n=1), *Alcaligenes xylosoxidans* (n=1), *Alcaugenes xylosoxidans* (n=1).

[d] Including *Chryseobacterium meningosepticum* (n=3), *Klebsiella oxytoca* (n=2), *Serratia marcescens* (n=2), *Enterobacter aerogenes* (n=1), *Burkholderia pickettii* (n=1), *Ralstonia pickettii* (n=1), *Acinetobacter pittii* (n=1), *Sphingomonas paucimobilis* (n=1), *Pseudomonas putida* (n=1), *Pseudomonas fluorescens* (n=1), *Mucor* (n=1).

Table 4. Multivariate logistic regression analysis of risk factors for death of Avian influenza A(H7N9) Virus in 350 Hospitalized Patients

Variable	**OR (95% CI)**	***P* Value**
Age	1.030(1.010-1.049)	**0.002**
Sex	0.741(0.473-1.257)	0.267
Current smoking	1.189(0.656-2.158)	0.568
Hypertension	0.937(0.535-1.640)	0.820
Heart diseases	0.783(0.349-1.758)	0.553
Diabetes	1.706(0.912-3.190)	0.095
COPD	1.162(0.465-2.899)	0.748
Time from illness onset to antiviral therapy initiation in d	1.069(1.003-1.139)	**0.040**
Secondary infection	1.978(1.214-3.223)	**0.006**

Abbreviations: OR, odds ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease.

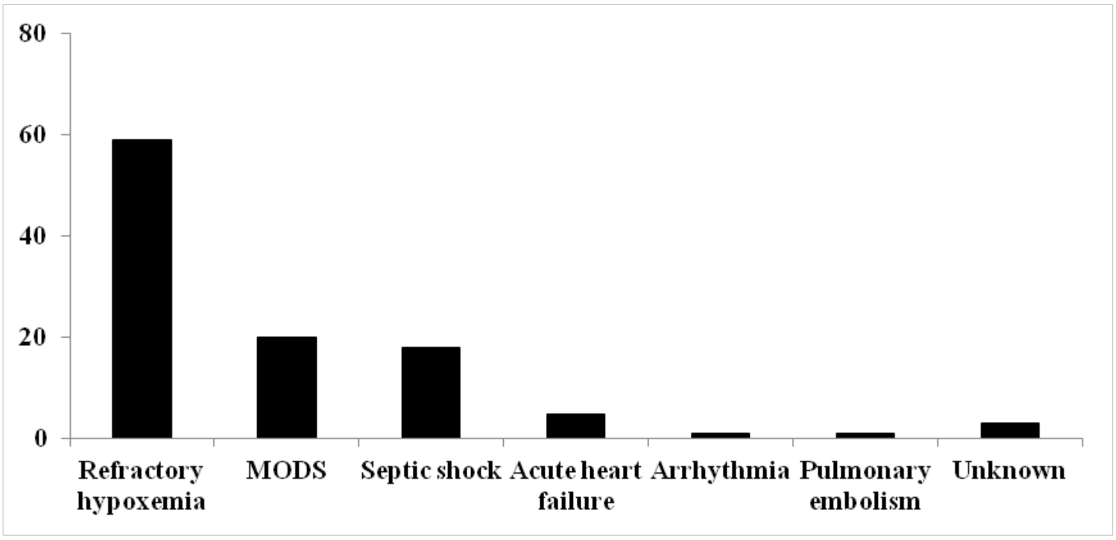

Figure 1. The cause of death in patients with H7N9 virus infection

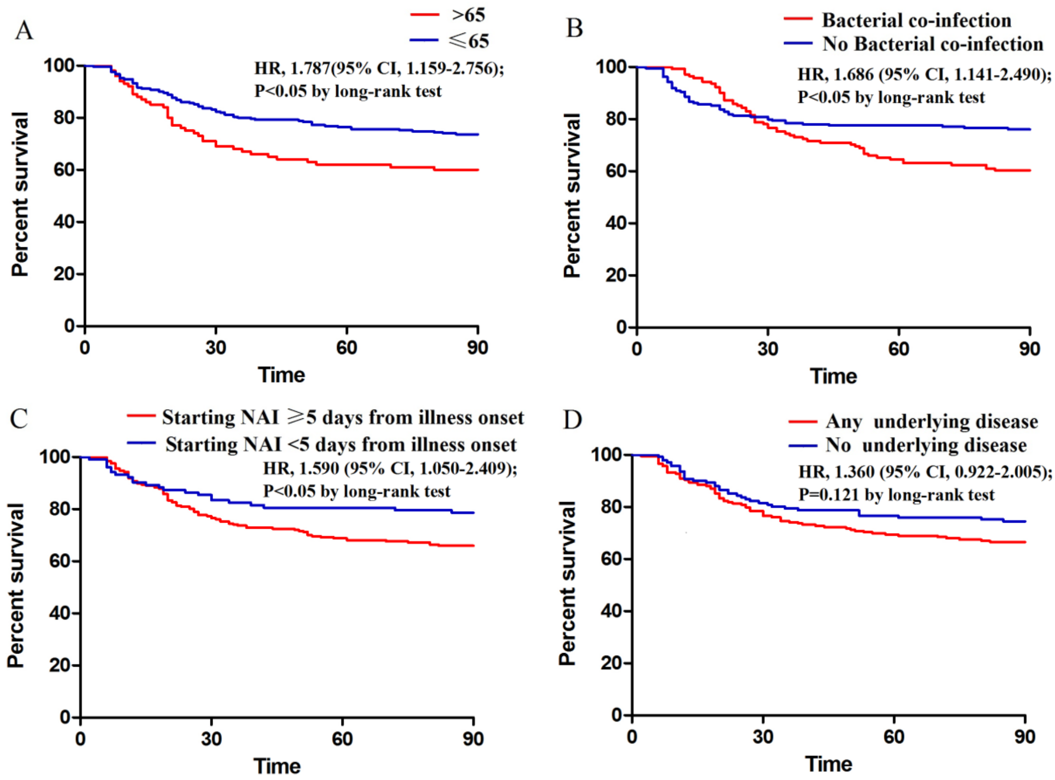

Figure 2. Kaplan–Meier survival curves of patients hospitalized for confirmed H7N9 influenza virus infections, censored at 90 days. Survival according to A) Age over 65 (log-rank test p $p<0.05$), B) Secondary bacterial infections (log-rank test $p<0.05$), C) NAI therapy within 5 days from symptom onset (log-rank test $p<0.05$), D) Any underlying disease (log-rank test $p=0.121$). HR, hazard ratio; CI, confidence interval.