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Prognosis and survival of 128 patients with severe avian influenza A(H7N9) infection in Zhejiang province, China

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SUMMARY

No published studies have discussed details of the prognosis and survival of patients with severe avian influenza A(H7N9) infection. In this study we analysed 128 laboratory-confirmed cases of severe H7N9 infection in Zhejiang province, the most affected region during the H7N9 epidemic in mainland China. We found that an increase in patient age by 5 years was associated with a 1·41 [95% confidence interval (CI) 1·19–1·67] times odds ratio for fatality. In addition, the time interval between the first clinical visit after symptom onset and hospital admission was inversely associated with survival time since admission. Of the 47 patients who died of the disease, when the time interval between the first clinical visit and hospital admission increased by 1 day, the duration of survival was 0·78 times (95% CI 0·62–0·98) as long. Our results suggest that patients with severe influenza H7N9 infection at older ages were at a higher risk of fatality, and that a delay in hospital admission was associated with more rapid death. More studies are required to corroborate our major findings.

Key words: Avian influenza A (H7N9), prognosis, survival.

INTRODUCTION

The outbreak of human infections involving avian influenza A(H7N9) virus in mainland China has tremendous public health significance because this virus had not previously been detected in humans [1]. By 5 June 2014, 14 provinces had reported a total of 433 laboratory-confirmed H7N9 cases, resulting in 164 deaths. The most affected region of this prolonged H7N9 epidemic was the Yangtze River Delta in eastern China [1], with the maximum number of patients identified in Zhejiang province: up to 11 April 2014, 138 cases, including 47 deaths, were reported. The most prominent feature of the H7N9 epidemic in Zhejiang was that all reported cases clustered in two calendar periods. The first wave of cases started at the very beginning of April 2013, after which the outbreak quickly ceased in the latter half of the month, with a total of 45 confirmed patients. The second wave stretched from October 2013 to the end of February 2014, with another 93 cases reported.

Since the outbreak, a number of epidemiological studies on factors associated with H7N9 incidence have been reported, revealing that older age and male sex were independent risk factors [2, 3]. Some researchers studied the prognosis of patients diagnosed with the disease, and found that factors such as age, smoking, underlying medical conditions, and

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certain serum parameters, including C-reactive protein level, myoglobin level, viral load, and lymphocyte count, were associated with fatality in patients with H7N9 [3–10]. However, nearly all studies of the prognosis of H7N9 infection have been based on a small number of patients, and therefore the reliability and validity of the results could be questioned. Moreover, no study had discussed the prognosis of patients with severe disease, or explored factors associated with duration of survival in patients with fatal infection. This study, using the most recent data, aimed to address these inadequacies.

METHODS

Case definition

According to the 'Guideline on diagnosis and treatment of human infection of H7N9 avian influenza (2014 edition)', which was issued by the National Health and Family Planning Commission (NHFPC) of the People's Republic of China [11], with the exception of laboratory confirmation, patients with severe H7N9 infection should meet at least one of the following criteria:

(1) Chest X-ray revealing multi-lobe pathological changes of the lung, or the progression of lung lesions by more than 50% within the last 48 h.
(2) Dyspnoea, with a respiratory rate greater than 24/min.
(3) Severe hypoxemia: under 3–5 l/min oxygen flow, a pulse oxygen saturation (SpO_2) of no more than 92%.
(4) Shock, adult respiratory distress syndrome (ARDS), or multiple organ dysfunction syndrome (MODS) presented.

Data collection

Based on the requirements of 'Outbreak prevention and control measurements of human infection of H7N9 avian influenza (third edition)', which was also formulated and implemented by NHFPC [12], all patients with laboratory-confirmed H7N9 infection were subjected to a standardized, questionnaire-based field epidemiological investigation. This investigation was performed by the jurisdictional county CDC. The main information collected included: general characteristics of the patients, history of disease onset, clinical visits, clinical manifestations, laboratory test results, diagnosis and prognosis, environmental features of the patient's residence, exposure history, and close contacts.

Under the mandate of the 'Law on Prevention and Control of Infectious Diseases' of The People's Republic of China, all investigations on human H7N9 infection, which is a listed notifiable disease, are exempt from ethical approval and informed consent.

Laboratory tests

Initially, for patients with suspected H7N9 infection, respiratory tract specimens were collected for real-time PCR (rtPCR) testing. RNA was extracted from the specimens using a Qiagen RNeasy mini-kit (Qiagen Inc., USA), and the use of H7N9-specific primers and probes according to the manufacturer's protocol. For those with a positive PCR test result, viral isolation was performed: specimens were injected into the allantoic cavities of pathogen-free embryonated chicken eggs. We performed rtPCR in bio-safety level (BSL)-2 facilities, and viral isolation in enhanced BSL-3 facilities, all located in Zhejiang Provincial Center for Disease Control and Prevention.

Statistical analysis

Descriptive statistics were used to illustrate general features of patients with severe H7N9 infection. Inter-subgroup differences were examined by t test, Kruskal–Wallis test or Fisher's exact test as appropriate to the specific variable. The prognosis of patients was categorized as 'fatal' or 'non-fatal'. As stated above, previous studies have revealed that age, sex, underlying medical conditions, antiviral therapy, and lymphocyte count were associated with death in patients with H7N9 infection. In addition, the first recorded body temperature after symptom onset and the time interval between symptom onset and the first clinical visit can represent the acuteness of disease. The white blood cell (WBC) count is a sensitive index of the immune response, and time interval between the first clinical visit and hospital admission can reflect the speed of disease progression. All these variables may be associated with prognosis and survival in patients with severe H7N9 infection. We used univariate and multivariate logistic regression models to estimate crude and adjusted odds ratios (ORs) of fatality for these variables. Survival time since hospital admission in patients with fatal disease

Table 1. *Demographic and clinical characteristics of 128 patients with severe influenza A(H7N9) infection, Zhejiang, China*

Characteristics	Overall ($N = 128$)	Fatal patients ($N = 47$)	Non-fatal patients ($N = 81$)	Statistic	P value
Age, years, mean (S.D.)	58·9 (14·4)	66·5 (11·9)	54·7 (13·8)	$t = 4·79$	<0·01*
Sex, male, *n* (%)	86 (67·2)	33 (70·2)	53 (65·4)	n.a.	0·70†
Occupation, *n* (%)				n.a.	0·09†
Farmer	60 (46·7)	22 (46·8)	38 (46·9)		
Homemaker or retired	40 (31·3)	19 (40·4)	21 (25·9)		
Others	28 (21·9)	6 (12·8)	22 (27·2)		
Comorbid hypertension, *n* (%)	49 (38·3)	18 (38·3)	31 (38·3)	n.a.	1·00†
Comorbid diabetes mellitus, *n* (%)	26 (20·3)	14 (29·8)	12 (14·8)	n.a.	0·06†
Comorbid cardiovascular disease, *n* (%)	12 (9·4)	2 (4·3)	10 (12·3)	n.a.	0·20†
Comorbid respiratory diseases, *n* (%)	6 (4·7)	2 (4·3)	4 (4·9)	n.a.	1·00†
Antiviral therapy: Yes, *n* (%)	102 (79·7)	37 (78·7)	65 (80·3)	n.a.	0·82†
Antiviral therapy in 48 h: Yes, *n* (%)	9 (7·0)	4 (8·5)	5 (6·17)	n.a.	0·72†
Symptom onset to first clinical visit, median (days)	1	1	1	$\chi^2 = 2·91$	0·09‡
First clinical visit to admission, median (days)	3	3	3	$\chi^2 = 0·91$	0·34‡
The first recorded body temperature, °C, mean (S.D.)	38·6 (0·9)	38·7 (0·8)	38·6 (0·9)	$t = 0·30$	0·76*
WBC count at admission 10^9/l, mean (S.D.)	4·9 (3·0)	5·5 (2·9)	4·7 (3·0)	$t = 1·39$	0·16*
Lymphocyte percentage at admission, %, mean (S.D.)	16·6 (8·7)	14·4 (9·8)	17·7 (7·9)	$t = 1·57$	0·12*

n.a., Not available; WBC, white blood cell.
† By Fisher's exact test.
‡ By Kruskal-Wallis test.
* By *t* test.

was logarithmically transformed to resemble a normal distribution, and univariate and multivariate linear regression models were similarly applied to calculate coefficients for relevant variables.

A few missing values were noted for several variables, including comorbidities [hypertension, diabetes mellitus (DM), cardiovascular diseases (CVD), respiratory diseases], WBC count, lymphocyte percentage, and the first recorded body temperature after symptom onset. A multiple imputation method was used to deal with this data incompleteness prior to model fitting. Variables with P values <0·10 in the univariate models were included in multivariate models. All statistical analyses were run using SAS v. 9·2 (SAS Institute Inc., USA). The significance level for statistical tests and inferences was defined as a two-tailed probability <0·05.

RESULTS

Until 11 April 2014, by applying the criteria stated above, we identified 128 patients with severe H7N9 infection in 138 laboratory-confirmed cases. The age of the patients ranged from 20 to 86 years (mean 58·9 years). The proportion of male patients was 67·2%, and the most common occupation was 'farmer' (46·7%). In total, 38·3% patients reported comorbid hypertension; 20·3%, 9·4%, and 4·7% reported comorbid DM, CVD, and respiratory diseases, respectively. The proportion of patients who received antiviral therapy was 80·0%, and 7·0% started antiviral therapy within 48 h after symptom onset. The median time intervals between symptom onset and the first clinical visit, and between the first clinical visit and hospital admission were 1 day and 3 days, respectively. The first recorded body temperature after symptom onset ranged from 36·4 °C to 41·0 °C. The mean WBC count and lymphocyte percentage at hospital admission were $4·9 \times 10^9$/l and 16·6%, respectively. Forty-seven patients died, with a case-fatality rate of 36·7%. Most demographic and clinical characteristics were comparable between patients with fatal and non-fatal disease, except for age, with a larger mean observed in patients who died (Table 1).

Two independent variables were identified and included in a multivariate logistic regression model: age and the time interval between symptom onset and the first clinical visit. However, only age was significantly associated with the prognosis of severe H7N9 infection: a 5-year increase in age was related to an increase of 0·41 (95% CI 1·19–1·67) in the odds of fatality (Table 2).

Table 2. *Logistic and linear regression results by different factors in 128 patients with severe influenza A(H7N9) infection, Zhejiang, China*

	Logistic regression model Event = fatality		Linear regression model Dependent = log(survival time since admission)	
Factors	Crude OR (95% CI)	Adjusted OR (95% CI)	Coefficient (S.E.)	Adjusted coefficient (S.E.)
Age (+5 years)	1·39 (1·19–1·64)**	1·41 (1·19–1·67)**	0·03 (0·05)	
Sex (contrast: male), female	0·80 (0·37–1·74)		0·12 (0·27)	
Occupation (contrast: farmer), others	1·00 (0·49–2·06)		0·25 (0·25)	
Any comorbidity (contrast: no)	2·29 (0·87–6·01)		0·31 (0·29)	
Antiviral therapy (contrast: no)	0·91 (0·38–2·21)		0·14 (0·33)	
Antiviral therapy within 48 h (contrast: no)	1·41 (0·36–5·55)		0·41 (0·43)	
Symptom onset to first clinical visit (+1 day)	0·83 (0·68–1·03)	0·80 (0·63–1·02)	0·12 (0·08)	
First clinical visit to admission (+1 day)	0·95 (0·82–1·11)		−0·12 (0·05)*	−0·11 (0·05)*
The first recorded body temperature (+1 °C)	1·21 (0·67–2·18)		0·03 (0·09)	
WBC count at admission (+1 × 10^9/l)	1·08 (0·96–1·22)		0·00 (0·01)	
Lymphocyte percentage at admission (+5%)	1·06 (0·90–1·26)		−0·05 (0·03)	−0·04 (0·03)

OR, Odds ratio; CI, confidence interval; WBC, white blood cell.
* $P < 0·05$, ** $P < 0·01$.

The Shapiro–Wilk test revealed that, after logarithmic transformation, the survival time since hospital admission of patients who died conformed to a normal distribution ($W = 0·97$, $P = 0·30$); the logarithmic mean of the survival time was 14·4 days. Two independent variables were identified and included in a multivariate linear regression model: the time interval between symptom onset and the first clinical visit, and the time between the first clinical visit and hospital admission. The multivariate model suggested that only the adjusted coefficient of the latter was significantly related to survival time since hospital admission. When the time interval between the first clinical visit and hospital admission increased by 1 day, the duration of survival was 0·78 times (95% CI 0·62–0·98) as long (Table 2).

DISCUSSION

The proportion of patients with severe H7N9 infection in those infected with the virus was 92·8% (128/138), much higher than reported by Ke *et al.* (72·4%) [13]. This observation emphasizes the severity of H7N9 virus infection in humans. The fatality rate in patients with severe H7N9 infection was 36·7%, comparable to that in a previous study of 123 hospitalized patients with H7N9 infection in China [14]. Compared with influenza H5N1, which has a reported fatality rate of 70% in hospitalized patients, a moderate risk of death was observed.

Using a multivariate logistic regression model, we found that age was significantly associated with fatality in patients with severe H7N9 infection: a 5-year increase in age was related to 40% higher odds of death. Similarly, two previous studies have observed an increased risk of serious illness or death in older patients with H7N9, and it was thought that this association could be partly ascribed to age-related confounders such as immunological status, comorbidities, and severity of disease [4, 6]. However, our study controlled for disease severity (all patients included had severe disease), adjusted for co-existing comorbidities, and precluded the influence of immunological status because neither WBC count nor lymphocyte percentage at admission was associated with H7N9 fatality in the univariate model. Therefore, the factors that contribute to the increased risk of fatality in older patients with severe H7N9 infection should be explored further.

Survival time since hospital admission is closely related to the final outcome of H7N9 infection, because a longer survival time increases the likelihood of cure. We found that survival time since hospital admission in patients with severe H7N9 infection was inversely associated with the time interval between the first clinical visit and admission: a 1-day delay in admission resulted in a 22% reduction in survival time. Given that no existing literature has discussed this topic, we hypothesize two possible explanations. First, the timely adoption of systematic, comprehensive and intensive medical treatment is critical to the survival of patients with severe H7N9 infection. Because patients can only receive this kind of treatment once they have been hospitalized, it is reasonable

to assume that a greater delay in admission will be associated with a poor outcome. Second, because the time interval between the first clinical visit and admission can reflect the speed of disease progression, we also suspect, somewhat counterintuitively, that slow progression in the early phase of the disease predicts expedited death.

Two previous studies have reported that underlying medical conditions, such as hypertension and chronic pulmonary disease, were associated with poor outcome in patients with H7N9 infection [4, 6]. In our study, we found that comorbidity in general was not associated either with fatality in patients with severe H7N9 infection, or with survival time since admission in patients who died. We believe the major reason for this discrepancy is that in the two studies cited, perhaps because of sample size limitations, the authors did not adjust for the influence of other potential confounders by using a multivariate method when estimating the ORs for underlying medical conditions. Therefore, it is possible that the association they identified was merely caused by age confounding, because older age is not only associated with a higher prevalence of comorbidity, but also with an increased risk of severe H7N9 infection [3].

Interestingly, we found that the adoption of antiviral therapy during the whole therapeutic process, or within 48 h after symptom onset, was not significantly related to either general prognosis or survival time since admission in patients with severe H7N9 infection. This result contrasts with two previous studies, which found that the use of antiviral therapy was associated with a better prognosis [4, 9]. As these two studies included both mild and severe cases, this discordance perhaps suggests that the effect of antiviral therapy on disease prognosis is only prominent in mild H7N9 cases. The 'National treatment guideline on H7N9 human infection' issued by NHFPC suggests that antiviral therapy should be implemented within 48 h of symptom onset. Considering the lack of influence of antiviral therapy on the prognosis and survival of patients with severe H7N9 infection found in the present study, and the predominance of side-effects associated with oseltamivir, the antiviral drug most commonly used for treatment of avian influenza [15], the use of antiviral therapy should be validated separately in H7N9 patients of different severity levels.

Our study has certain limitations. First, some information regarding the patients was collected by retrospective interview, thus the potential for recall bias cannot be ignored. Second, because of individual differences in disease tolerance, a single index, such as the first recorded body temperature after symptom onset, may correspond to different stages of disease in different patients, and this will introduce bias to the study results. Finally, we used a multiple imputation method to deal with a small amount of missing data, if the data were missed non-randomly, our study result will be further biased.

Despite the limitations stated above, our study identified factors associated with prognosis and survival in patients with severe H7N9 infection for the first time. The results suggest that more intense treatment should be administered to older patients with severe H7N9 infection. Other major findings, such as the association of a delay in hospital admission with expedited death, and the differing effect of antiviral therapy in mild and severe cases, should be corroborated by future studies.

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DECLARATION OF INTEREST

None.

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