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Factors Associated With Prolonged Viral Shedding in Patients With Avian Influenza A(H7N9) Virus Infection

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Background. Data are limited on the impact of neuraminidase inhibitor (NAI) treatment on avian influenza A(H7N9) virus RNA shedding.

Methods. In this multicenter, retrospective study, data were collected from adults hospitalized with A(H7N9) infection during 2013–2017 in China. We compared clinical features and A(H7N9) shedding among patients with different NAI doses and combination therapies and evaluated factors associated with A(H7N9) shedding, using Cox proportional hazards regression.

Results. Among 478 patients, the median age was 56 years, 71% were male, and 37% died. The median time from illness onset to NAI treatment initiation was 8 days (interquartile range [IQR], 6–10 days), and the median duration of A(H7N9) RNA detection from onset was 15.5 days (IQR, 12–20 days). A(H7N9) RNA shedding was shorter in survivors than in patients who died ($P < .001$). Corticosteroid administration (hazard ratio [HR], 0.62 [95% confidence interval {CI}, .50–.77]) and delayed NAI treatment (HR, 0.90 [95% CI, .91–.96]) were independent risk factors for prolonged A(H7N9) shedding. There was no significant difference in A(H7N9) shedding duration between NAI combination treatment and monotherapy ($P = .65$) or between standard-dose and double-dose oseltamivir treatment ($P = .70$).

Conclusions. Corticosteroid therapy and delayed NAI treatment were associated with prolonged A(H7N9) RNA shedding. NAI combination therapy and double-dose oseltamivir treatment were not associated with a reduced A(H7N9) shedding duration as compared to standard-dose oseltamivir.

Keywords. Viral shedding; avian influenza A(H7N9); human; antiviral drug; neuraminidase inhibitors; oseltamivir; corticosteroid; risk factor.

Since the first 3 patients known to have avian influenza A(H7N9) virus infection were reported in March 2013 [1], there have been 5 epidemics of A(H7N9) infections among humans to date in mainland China [2]. In these 5 epidemics, most A(H7N9)-infected patients were hospitalized with severe illness, with a high resultant mortality rate (approximately 40%) [2]. During the fifth epidemic, a sudden increase in the number of human cases and the emergence of highly pathogenic A(H7N9) infections raised public health concerns [3–7].

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Factors contributing to the pathogenesis of influenza virus infection include direct injury caused by infection and viral replication in respiratory epithelial cells and immunopathology induced by cytokine dysregulation [8, 9]. Observational studies have reported that prolonged influenza viral shedding in the respiratory tract is associated with severe outcomes of patients with seasonal influenza [10] and that early initiation of antiviral treatment with neuraminidase inhibitors (NAIs) for patients infected with 2009 pandemic influenza A(H1N1) virus (A[H1N1] pdm09) has a survival benefit [11–17]. The viral shedding duration among patients with seasonal or pandemic influenza has been reported to be associated with corticosteroid administration, delayed NAI treatment, and comorbidities [10, 18].

NAI treatment of A(H7N9)-infected patients in China has included standard- or double-dose oseltamivir monotherapy, as well as combination NAI treatment with oseltamivir and peramivir. Corticosteroids have also been administered to most A(H7N9)-infected patients. Recently, a single institution study reported that A(H7N9) viral shedding duration was shorter and that mortality was lower with early initiation of NAI treatment [19]. However, the study did not assess the impact of different NAI drug doses, combination NAI treatment, or corticosteroid therapy on the duration of A(H7N9) shedding.

We conducted a retrospective, multicenter study of 478 hospitalized patients with laboratory-confirmed A(H7N9) infection who were identified during April 2013–March 2017 to assess the impact of different NAI treatment strategies and corticosteroid treatment on the duration of A(H7N9) shedding.

METHODS

Data Collection

Data collection and analysis were coordinated by the Chinese National Health and Family Planning Commission. The subjects included in the study were patients with laboratory-confirmed A(H7N9) infection diagnosed from 1 April 2013 to 1 March 2017. The study subjects were expanded from the cohort of 288 hospitalized A(H7N9)-infected patients with pneumonia during April 2013–March 2015 that was previously analyzed to assess the impact of corticosteroids upon mortality and A(H7N9) shedding [20]. The electronic medical records of patients were sent to a data collection center in Beijing, China. Demographic data, comorbidities, vital signs, laboratory findings at admission, virological test results, and treatments were reviewed by a trained team of physicians and medical students and entered in duplicate into a computerized database. As noted previously, the collection of data from A(H7N9)-infected patients was determined to comprise public health surveillance in China, and institutional review board assessment was waived [20, 21].

Virologic Investigations

A(H7N9) infection was confirmed in all patients by testing respiratory specimens with a real-time reverse transcription–polymerase chain reaction (RT-PCR) assay at the local laboratory of the Chinese Center for Disease Control and Prevention. Respiratory specimens (nasopharyngeal swab specimens, sputum specimens, endotracheal aspirates, or bronchoalveolar lavage fluid) were collected daily for detection of A(H7N9) RNA by real-time RT-PCR. Details of laboratory confirmation of A(H7N9) were described previously [21]. Respiratory specimens with cycle threshold (Ct) values of ≤38 were considered positive for A(H7N9), and those with Ct values of >38.0 underwent repeat testing. Upon repeated testing, respiratory specimens with Ct values of ≤38 were considered positive for A(H7N9), and those with Ct values of >38 or with undetectable results were considered negative for A(H7N9). We defined the interval between symptom onset and the date of the last A(H7N9) RNA–positive result for respiratory samples as the A(H7N9) RNA shedding duration. Corticosteroid treatment was defined as administration of a dose equivalent to ≥25 mg of methylprednisolone per day during hospitalization [22].

Statistical Analysis

Mean values and standard deviations or median values with interquartile range were used to describe continuous variables, and absolute or relative frequencies were used to describe categorical variables. We used the Student *t* test and the Mann-Whitney *U* test for analysis of continuous variables and the χ^2 test or Fisher exact test for analysis of discrete variables in bivariate analyses. To identify risk factors associated with prolonged duration of A(H7N9) RNA shedding, we performed a time-dependent Cox proportional hazards model that adjusted for baseline covariates. Outcomes were defined as times to A(H7N9) RNA negativity. For this analysis, we censored patients if they never cleared A(H7N9) RNA or, if they were discharged alive before they had cleared A(H7N9) RNA, at hospital discharge. Analysis was performed using a marginal structural Cox proportional hazard model [23]. Candidate variables considered for analysis of prolonged duration of A(H7N9) shedding were sex, age, comorbidities, smoking, time from illness onset to starting antiviral treatment, and specific antiviral NAI. We used Kaplan-Meier survival analysis to estimate the cumulative A(H7N9) RNA–negativity rate and the stratified log-rank statistic to compare the difference of A(H7N9) clearance between different 2 groups (ie, patients who survivors vs those who died, patients who started NAI treatment <5 days from illness onset vs those who started NAI treatment ≥5 days from illness onset, patients who received oseltamivir vs those who received a combination of oseltamivir plus peramivir, and patients who received standard-dose oseltamivir vs those who received double-dose oseltamivir). Statistical analyses were performed using SPSS, version 20.0 for Windows (IBM, Armonk, NY), and SAS, version 9.4 (SAS Institute, Cary, NC). For all analyses, probabilities were 2-tailed, and a 2-tailed *P* value of <.05 was considered significant.

RESULTS

From 1 April 2013 to 1 March 2017, 478 A(H7N9) case patients with virological results were included in this study from 16 provinces and 3 municipalities in mainland China. A total of 88 patients (18.4%) who tested positive for A(H7N9) RNA until death, and 390 (81.6%) had resolution of A(H7N9) shedding (Figure 1). The main characteristics and outcomes of all patients are summarized in Table 1.

The source of respiratory specimens was available in medical records for 244 patients (51%) for detection of the first negative A(H7N9) RNA result. Of the specimens tested that yielded the first negative A(H7N9) RNA test result, 145 (59%) were from an endotracheal aspirate, 35 (14.3%) were from a sputum specimen, and 64 (26.2%) were from a nasopharyngeal swab specimen.

Prolonged Duration of A(H7N9) RNA Shedding

The median duration of A(H7N9) RNA shedding was 15.5 days (interquartile range [IQR], 12–20 days). Only 7 patients (1.8%) with real-time RT-PCR confirmed A(H7N9) infection had undetectable A(H7N9) RNA within 6 days, 42 (10.7%) tested negative within 10 days, and 286 (73.1%) tested negative within 20 days of illness onset. A(H7N9) RNA was undetectable among most patients (92.3%) within 27 days after symptom onset, but it was detectable in a small subset of 22 patients up to 30 days after symptom onset (Figure 2 and Supplementary Table 1).

The median duration of A(H7N9) shedding detected in endotracheal aspirate specimens was 17 days (IQR, 13–21 days) and was similar to the median duration of A(H7N9) shedding detected in nasopharyngeal swabs (17 days [IQR, 13–20 days]; hazard ratio [HR], 1.18 [95% confidence interval {CI}, .78–1.79; P = .43; Supplementary Figure 1). Viral RNA clearance was significantly faster in survivors compared with fatal cases (HR, 1.79 [95% CI, 1.45–2.20]; P < .001; Figure 3A).

Risk Factors for Prolonged Viral Shedding

In a multivariable model that included available data from 478 patients, the time from illness onset to antiviral treatment (HR, 0.90 [95% CI, .91–.96]) and systemic corticosteroid administration (HR, 0.62 [95% CI, .50–.77]) were independent factors associated with the duration of A(H7N9) RNA shedding (Table 2 and Figure 4). Similar risk factors for delayed A(H7N9) RNA clearance were observed when the data were analyzed using a marginal structural Cox proportional hazards model (Supplementary Table 2).

The median time from illness onset to NAI treatment initiation was 8 days (IQR, 6–10 days). A(H7N9) RNA clearance was significantly delayed in patients who received NAI treatment beginning ≥5 days after illness onset, compared with those in whom NAI treatment was started <5 days after illness onset (HR, 2.64 [95% CI, 1.74–4.03]; P < .001; Figure 3B).

Impact of Different Antiviral Treatment Regimens and Oseltamivir Dosing on Outcomes

Among the 478 patients, antiviral treatment administered included oseltamivir monotherapy in 307 (64.2%); peramivir and oseltamivir in 142 (29.7%); peramivir, zanamivir, and oseltamivir in 25 (5.2%); oseltamivir and ribavirin in 3 (0.6%); and oseltamivir and ganciclovir in 1 (0.2%). The median duration of A(H7N9) shedding was 17 days (range, 13–21 days) in the oseltamivir monotherapy group and 19 days (range, 14–22 days) in the oseltamivir-peramivir combination treatment group. The main characteristics of patients who received different antiviral drugs are summarized in Table 3. The median time from antiviral treatment initiation to a negative real-time RT-PCR result in respiratory tract specimens was 8 days (range, 5–10 days) in the oseltamivir monotherapy group, and 8 days (range, 6–9 days) in the oseltamivir-peramivir combination therapy group (P = .76). There was no significant difference in duration of A(H7N9) RNA shedding between patients who received oseltamivir

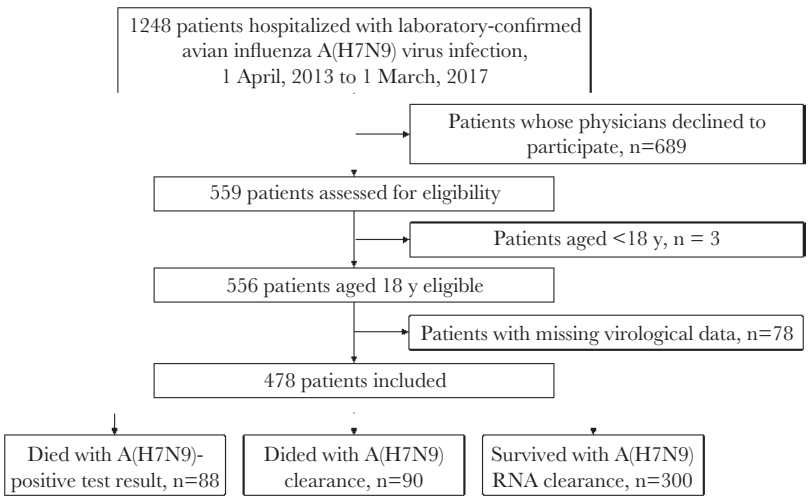

Figure 1. Flow diagram of hospitalized patients with confirmed avian influenza A(H7N9) virus infection included into this study.

Table 1. Characteristics of 478 Hospitalized Patients With Avian Influenza A(H7N9) Virus Infection in China From 1 April 2013 to 1 March 2017

Characteristic	Value
Age, y	56 (45–66)
Male sex	340 (71.1)
Comorbidity	
Hypertension	158 (33.1)
Diabetes	67 (14.0)
Cardiac disease[a]	44 (9.2)
COPD	20 (4.2)
Chronic renal insufficiency	17 (3.6)
Immunosuppression[b]	7 (1.5)
Severity score and laboratory finding on admission	
SOFA score	5 (3–6)
White blood cell count, $\times 10^9$ cells/L	4.3 (2.9–6.9)
Lymphocyte count, $\times 10^9$ lymphocytes/L	0.5 (0.3–0.7)
Platelet count, $\times 10^9$ platelets/L	130 (93–172)
Creatinine level, µmol/L	74.0 (57.0–98.0)
PaO_2/FiO_2, mm Hg	120 (76–199)
AST level, U/L	70 (42–130)
Creatine kinase level, U/L	290 (102–750)
Time to virological result, d	
From illness onset to A(H7N9) infection diagnosis	8 (6–10)
From A(H7N9) infection diagnosis to ART start	1(0–1)
From symptom onset to ART start	8 (6–10)
Viral shedding duration[c]	15.5 (12–20)
Treatment or complication	
Corticosteroid therapy	346 (72.4)
Septic shock	166 (34.7)
ICU admission	355 (74.3)
Invasive mechanical ventilation	249 (52.1)
ECMO	64 (13.4)
CRRT	89 (18.6)
Outcome	
ICU length of stay, d	13 (7–25)
Hospital length of stay, d	18 (11–27)
In-hospital mortality	178 (37.2)

Continuous variables are expressed as median values (interquartile ranges), and categorical variables are presented as number of patients (percentages). Normal ranges and definition of abnormal values for white blood cell count, lymphocytes, platelets, creatinine, PaO_2/FiO_2, AST, and creatine kinase are listed in the Supplementary Tables and Figures.

Abbreviations: ARV, antiviral treatment; AST, aspartate aminotransferase; COPD, chronic obstructive pulmonary disease; CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; FiO_2, fraction of inspired oxygen; ICU, intensive care unit; NAI, neuraminidase inhibitor; PaO_2, partial pressure of oxygen; SOFA, sequential organ failure assessment.

[a]Includes congestive heart disease and coronary atherosclerotic heart disease.

[b]Defined as receipt of chemotherapy or radiotherapy within 1 month before the onset of illness or receipt of corticosteroid therapy (equivalent of 30 mg of prednisone per day) for 15 continuous days before the onset of illness.

[c]Data are based on real-time reverse transcription–polymerase chain reaction analysis.

monotherapy, compared with those who received oseltamivir-peramivir combination therapy (Figure 3C).

Oseltamivir dosing data were available for 180 patients. There was no difference in A(H7N9) RNA clearance (HR, 1.08 [95% CI, .67–1.68]; $P = .7$; Figure 3D) or in-hospital mortality (51.4% and 57.5%, respectively; $P = .58$; Table 3) between patients who received oseltamivir 150-mg twice-daily treatment and those who received 75-mg twice-daily treatment.

We further compared the proportion of patients who tested positive for A(H7N9) RNA over time after starting NAI treatment, using the log-rank test. There was no significant difference among patients who received standard-dose oseltamivir, double-dose oseltamivir, or oseltamivir and peramivir treatment (HR, 1.18 [95% CI, .78–1.79]; $P = .43$; Supplementary Figure 2).

DISCUSSION

We report on the largest cohort of hospitalized patients with A(H7N9) infection in China to date. This study expands on our prior study of 288 hospitalized A(H7N9)-infected patients with pneumonia that reported associations between high-dose corticosteroid therapy and increased 30-day and 60-day mortality and a longer duration of A(H7N9) RNA detection [20]. In the current study of 478 hospitalized A(H7N9)-infected patients, we identified independent risk factors for prolonged A(H7N9) RNA detection and examined the impact of different antiviral treatment regimens on A(H7N9) RNA shedding. In most patients, NAI treatment was started >48 hours after illness onset, but initiation of NAI treatment before the fifth day of illness was associated with a shorter duration of A(H7N9) RNA shedding. Combination antiviral treatment with different NAIs and double-dose oseltamivir was not associated with significant differences in A(H7N9) RNA shedding or in-hospital mortality, compared with standard-dose oseltamivir monotherapy.

We detected A(H7N9) RNA in the respiratory tract for a median of 15.5 days, and prolonged A(H7N9) RNA shedding was associated with a fatal outcome. Data on the duration of highly pathogenic avian influenza A(H5N1) virus shedding are limited but indicate that A(H5N1) RNA can be detected longer and at higher levels in lower respiratory tract tissues, including up to 15–27 days in fatal cases, than in upper respiratory tract specimens [24, 25]. A systematic review reported that progressively longer shedding duration of A(H1N1)pdm09 in patients was correlated with disease severity when studies were stratified into community (range of mean and median values, 2–9 days), hospital (range of mean and median values, 6–10 days), and intensive care (range of mean and median values, 13–20 days) settings [18]. We detected A(H7N9) RNA for up to 30 days in 22 patients, most of whom received a corticosteroid dose of at least 40 mg/day (50 mg prednisone/day), which can be considered an immunosuppressive dose (Supplementary Table 1). Influenza viral RNA can be detected in the respiratory tract, particularly the lower respiratory tract, for a prolonged period in patients with severe disease or immunosuppression after illness onset, and better understanding of the kinetics of influenza virus replication in the upper and lower respiratory tracts of A(H7N9)-infected patients would be useful in future studies.

Delayed initiation of NAI treatment was an independent risk factor associated with prolonged A(H7N9) RNA shedding. Similarly, in a single-center retrospective study, Zheng et al reported that A(H7N9) RNA shedding was significantly shorter

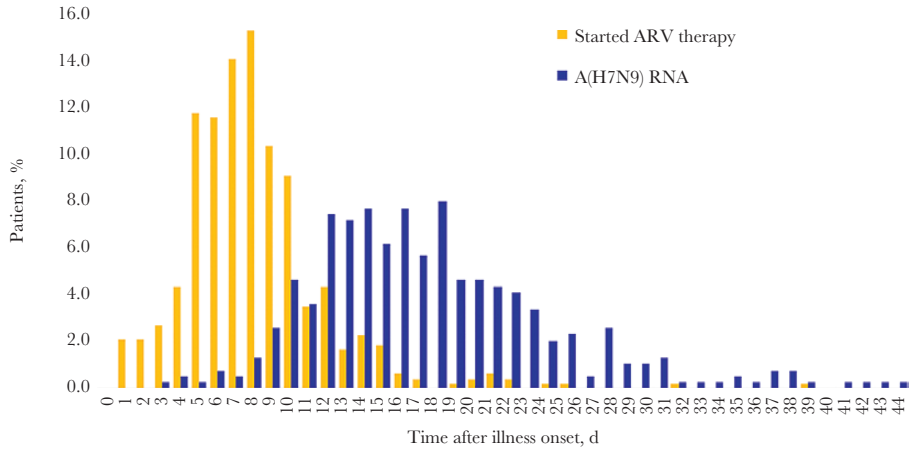

Figure 2. Distribution of antiviral treatment (ARV) and proportion of patients with undetectable avian influenza A(H7N9) virus RNA by day after onset of symptom

when NAI treatment was started within 2 days of illness onset than when antiviral treatment was started later [19]. The median time from illness onset to NAI treatment initiation was 8 days among the 478 patients in our study. Earlier administration of NAI treatment might have reduced viral shedding and improved survival in these A(H7N9)-infected patients.

A large meta-analysis of individual-level data for patients hospitalized with A(H1N1)pdm09 infection reported that,

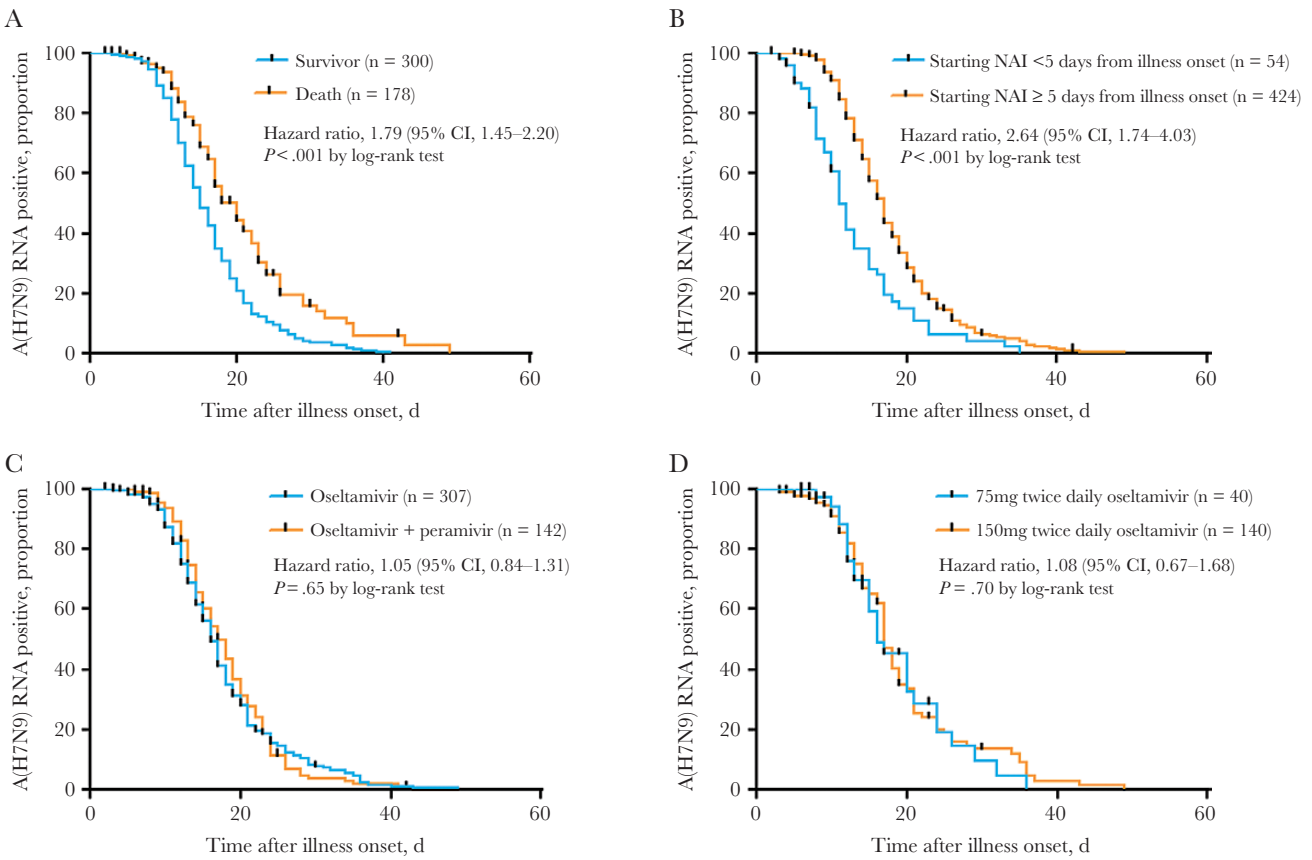

Figure 3. *A*, Cumulative proportion of patients between patients who survived and those who died with detectable avian influenza A(H7N9) virus RNA, by day after onset of illness. *B*, Cumulative proportion of patients who started neuraminidase inhibitor (NAI) therapy <5 days versus ≥5 days after illness onset who had detectable A(H7N9) RNA, by day after onset of illness. *C*, Cumulative proportion of patients treated with oseltamivir versus oseltamivir and peramivir who had detectable A(H7N9) RNA, by day after onset of illness. *D*, Cumulative proportion of patients treated with oseltamivir (75 mg twice daily) versus oseltamivir (150 mg twice daily) with detectable A(H7N9) RNA, by day after onset of illness. CI, confidence interval.

Table 2. Multivariable Analyses of Factors Associated With Duration of Avian Influenza A(H7N9) Virus RNA Detection in 478 Hospitalized Patients From China, 1 April 2013–1 March 2017

Variable	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*[a]
Demographic characteristic				
Age	0.99 (.99–1.00)	.102	0.99 (.99–1.00)	.115
Male sex	0.98 (.80–1.25)	.981	1.02 (.81–1.27)	.879
Comorbidity				
Current smoking	1.05 (.83–1.33)	.661	…	
Pregnant	1.51 (.67–3.38)	.322	…	
Hypertension	1.02 (.82–1.26)	.884	…	
Heart diseases	0.89 (.63–1.28)	.533	…	
Diabetes	1.10 (.82–1.47)	.526	…	
COPD	1.0 (.60–1.68)	.997	…	
Chronic renal insufficiency	1.24 (.71–2.15)	.455		
Immunosuppression	2.40 (1.07–5.42)	.035	1.64 (.72–3.73)	.238
Drug treatment				
Corticosteroid	0.64 (.52–0.80)	<.001	0.62 (.50–.77)	<.001
Oseltamivir monotherapy at any dose	Reference		Reference	
Oseltamivir-peramivir combination	0.95 (.77–1.19)	.679	0.90 (.72–1.12)	.346
Oseltamivir-peramivir-zanamivir combination	1.12 (.76–1.66)	.572	0.99 (.67–1.47)	.958
Time from illness onset to ARV initiation in d	0.91 (.88–.93)	<.001	0.9 (.91–.96)	<.001

Abbreviations: ARV, antiviral therapy; CI, confidence interval; COPD, chronic obstructive pulmonary disease.

[a]By use of the time-dependent Cox proportional hazards model. A hazard ratio (HR) of <1 indicates that the variable increases the duration of A(H7N9) RNA shedding. HRs in multivariable analyses were adjusted for age and sex.

while early (≤2 days) versus later (3–5 days) initiation of NAI treatment was significantly associated with a survival benefit in adults, later initiation of NAI treatment was significantly associated with a lower mortality risk, compared with no treatment [17]. Our findings also suggest that NAI treatment should be started as early as possible if A(H7N9) infection is suspected, without waiting for virological confirmation.

Corticosteroid therapy is commonly administrated in critically ill patients with influenza to reduce cytokine dysregulation and lung inflammation and to improve clinical outcomes [26]. However, most observational studies have reported that use of corticosteroids was associated with persistent viral shedding in patients with seasonal influenza, Middle East respiratory syndrome, or severe acute respiratory syndrome [10, 23, 27]. In addition, our previous observational study that used propensity-score matching showed that use of high-dose corticosteroids was associated with a fatal outcome among patients with pneumonia and A(H7N9) infection [20]. Our results are in accordance with the above findings that corticosteroid treatment, not only high-dose corticosteroid dosing, is associated with prolonged viral shedding duration and that prolonged viral shedding is associated with a fatal outcome. The mechanisms of delayed viral clearance due to corticosteroid treatment are not well defined but might be associated with impaired T-cell responses [10]. Taken together, our findings suggest that corticosteroids should not be given for treatment of A(H7N9) infection.

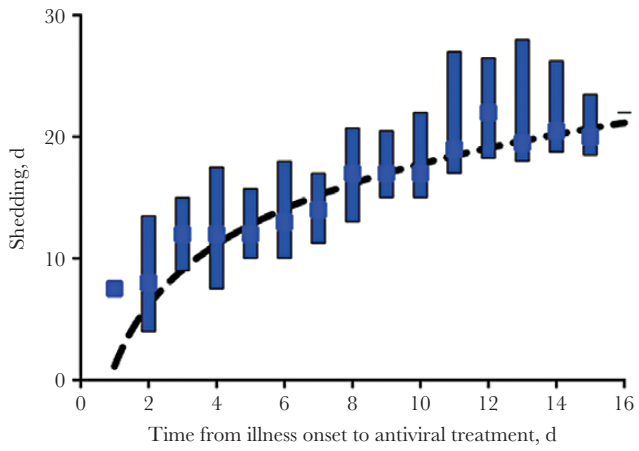

Figure 4. Duration of avian influenza A(H7N9) virus RNA detection in relationship to time from illness onset to antiviral treatment initiation.

Another potential reason for prolonged duration of viral RNA shedding is the emergence of NAI resistance during antiviral treatment. Hu et al [28] reported the emergence of oseltamivir-resistant virus with the Arg292Lys (R292K) mutation in the gene encoding viral neuraminidase in 2 A(H7N9)-infected patients during NAI treatment, which was temporally associated with a rebound in the A(H7N9) load and treatment failure. The R292K mutation confers resistance to oseltamivir and highly reduced inhibition to peramivir and zanamivir in vitro, suggesting that NAI treatment would not be effective in treating infection with viruses containing the R292K mutation. Of concern, the prevalence of the R292K mutation has increased since the first human infections with A(H7N9), in 2013, and was reported to be approximately 11% among sequenced viruses from A(H7N9)-infected patients during the fifth epidemic [4].

Table 3. Comparison of Characteristics of Hospitalized Patients With Avian Influenza A(H7N9) Virus Infection Treated With Combination and Different Doses of Antiviral Therapy (ARV), China, 1 April 2013–1 March 2017

Variables	Oseltamivir-Peramivir Combination (n = 142)	Oseltamivir (n = 307)	*P*	Oseltamivir 150 mg Twice Daily (n = 140)	Oseltamivir 75 mg Twice Daily (n = 40)	*P*
Demographic characteristic						
Age, y	56 (42–66)	57 (45–67)	.413	55 (44–65)	59 (48–66)	.347
Male sex	91 (64.1)	230 (74.9)	.024	101 (72.1)	32 (80)	.415
Comorbidity						
Hypertension	59 (41.5)	94 (30.6)	.025	34 (24.3)	11 (27.5)	.682
Heart disease	5 (3.5)	34 (11.1)	.007	13	4 (10)	1
Diabetes	28 (19.7)	35 (11.4)	.028	17 (12.1)	4 (10)	1
COPD	5 (3.5)	13 (4.2)	.802	7 (5)	0	.351
Chronic renal insufficiency	2 (1.4)	14 (4.6)	.107	8 (5.7)	1 (2.5)	.686
Immunosuppression	1 (0.7)	6 (2.0)	.44	3 (2.1)	0	1
Current smoker	32 (22.5)	70 (22.8)	1	22 (15.7)	14 (35)	.012
Pregnancy	1 (0.7)	6 (2.0)	.44	2 (1.4)	0	1
Signs or laboratory findings at admission						
Systolic blood pressure, mm Hg	120 (107–140)	126 (115–140)	.017	123 (110–140)	126 (117–139)	.534
PaO_2/FiO_2, mm Hg	112 (74–207)	118 (75–193)	.756	108 (64–186)	92 (71–193)	.874
WBC count, $\times 10^9$ cells/L	4.1 (2.8–6.8)	4.3 (2.8–6.8)	.915	4.4 (2.7–7.0)	3.7 (2.4–6.0)	.229
Lymphocyte count, $\times 10^9$ lymphocytes/L	0.51 (0.32–0.79)	0.48 (0.30–0.70)	.357	0.51 (0.35–0.80)	0.44 (0.29–0.66)	.35
Platelet count, $\times 10^9$ platelets/L	131 (92–176)	127 (91–1652)	.469	130 (102–165)	144 (87–196)	.341
Creatinine level, μmol/L	72.0 (57.8–98.0)	77.0 (57.5–102.3)	.574	85.0 (60.9–115.0)	82.2 (63.8–136.8)	.527
Creatine kinase level, U/L	240 (90–745)	322 (107–789)	.484	442 (153–95)	360 (134–947)	.614
AST level, U/L	70 (42–134)	73 (43–119)	.875	79 (49–149)	70 (37–157)	.563
Lactate dehydrogenase level, U/L	647 (431–905)	584 (400–940)	.323	673 (424–1124)	648 (378–962)	.59
Treatment and complication						
Antibiotic therapy	136 (95.8)	285 (99.7)	.393	136 (97.1)	40 (100)	.577
Corticosteroid therapy	103 (72.5)	220 (71.7)	.91	108 (77.1)	30 (75.0)	.833
Septic shock	48 (33.8)	110 (35.8)	.672	55 (39.3)	14 (35)	.714
Invasive mechanical ventilation	73 (51.4)	167 (54.4)	.543	87 (62.1)	22 (55)	.465
ECMO	19 (13.4)	47 (15.3)	.668	24 (17.1)	3 (7.5)	.207
CRRT	23 (16.2)	65 (21.2)	.251	34 (24.3)	8 (20)	.674
Time from illness onset to laboratory diagnosis, d	8 (6–10)	8 (6–11)	.818	8 (6–11)	9 (7–12)	.241
Time from illness onset to ART initiation, d	8 (6–9)	8 (5–10)	.761	8 (6–10)	7 (6–9)	.134
Duration of A(H7N9) RNA shedding, d	19 (14–22)	17 (13–21)	.131	17 (13–21)	16 (12–21)	.841
Outcome						
Hospital LOS, d	18 (11–29)	18 (10–26)	.474	17 (7–25)	14 (8–21)	.233
In-hospital mortality	47 (33.1)	125 (40.7)	.144	72 (51.4)	23 (57.5)	.58

Continuous variables are expressed as median values (interquartile ranges), and categorical variables are presented as number of patients (percentages).

Among the 478 patients, 307 (62.9%) received oseltamivir monotherapy, 142 (29.1%) received combination therapy with peramivir plus oseltamivir, and 29 (5.9%) who are not included in first 2 columns (19 [4.0%] received combination therapy with peramivir, zanamivir, and oseltamivir; 7 [1.4%] received combination therapy with oseltamivir plus ribavirin; 2 [0.4%] received combination therapy with oseltamivir plus ganciclovir; and 1 [0.2%] received peramivir only).

Of 269 patients during the fifth epidemic with data available on neuraminidase inhibitor dosing, 140 were administered standard-dose (75 mg twice daily) oseltamivir, and 40 received 150-mg twice-daily oseltamivir; the remaining 89 are not included in last 2 columns because they received combination therapy with other antiviral agents.

Abbreviations: AST, aspartate aminotransferase; COPD, chronic obstructive pulmonary disease; CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; FiO_2, fraction of inspired oxygen; LOS, length of stay; PaO_2, partial pressure of oxygen; WBC, white blood cell.

Recently, Ke et al [29] described the detection of the R292K mutation as early as 2 days after starting oseltamivir treatment in a patient with highly pathogenic A(H7N9) infection.

Our study is the first to assess the impact of different NAI regimens, including combination treatment, and different dosages of oseltamivir on viral shedding and clinical outcome in patients with A(H7N9) infection. Our finding that double-dose oseltamivir did not provide an additional virologic benefit as compared to standard-dose oseltamivir treatment is consistent with results from controlled trials in outpatients [30] and inpatients with seasonal influenza [31] and from observational studies in hospitalized patients with A(H5N1) [32] or A(H1N1) pdm09 [33] infections. Previous studies suggested reasons for these observations [31, 34]. Oseltamivir is generally well absorbed and rapidly converted to the active compound, oseltamivir carboxylate, with a bioavailability of >80% [35]. Blood

concentrations of oseltamivir carboxylate associated with single- or double-dose therapy in patients with influenza both exceed the median inhibitory concentration (ie, 0.7–2.2 ng/mL [36]) of influenza viruses. Exceeding these concentrations might not produce additional clinical or virological effect [37]. In addition, it may be that delayed antiviral treatment, when severe disease is already present, may reduce any potential benefit of double-dose oseltamivir. A retrospective analysis from a limited number of patients with A(H7N9) infection reported that oseltamivir plus peramivir was not superior to oseltamivir monotherapy in clinical or virological outcomes [38]. Clinical data, including a randomized trial of combination oseltamivir and zanamivir treatment, compared with either monotherapy alone, for seasonal influenza have not substantiated a potential advantage in using combinations of NAIs [30]. Rather, the data appear consistent in showing that use of combinations that target the same protein do not show an additive or synergistic effect against influenza virus infection [39].

This study has several limitations. First, our study was limited by its retrospective nature, with dosing of antivirals missing in some patients during the first 3 epidemics. Second, not all of the patients with laboratory-confirmed A(H7N9) infection in mainland China could be included in this study. However, we were able to include patients managed at 96 different hospitals. Third, owing to the small number of patients who initiated NAI treatment 2–3 days of illness onset, we were not able to assess the effect of early versus later initiation of NAI treatment. Fourth, genetic sequencing was not performed on specimens from patients with prolonged duration of A(H7N9) RNA shedding, to assess the frequency of emergence of antiviral resistance. It was therefore not possible to analyze the relationship between antiviral resistance and prolonged A(H7N9) shedding. Since paired upper and lower respiratory tract specimens were not collected prospectively from all patients, we could not directly compare differences in viral shedding between upper (ie, nasopharyngeal swab specimens) versus lower (endotracheal aspirates) respiratory tract specimens. However, we did not find any significant difference in the duration of A(H7N9) shedding between nasopharyngeal swab specimens and endotracheal aspirates.

In conclusion, prolonged A(H7N9) RNA shedding in the respiratory tract was independently associated with delayed initiation of NAI treatment and with use of corticosteroids. These results reinforce guidance that NAI treatment should be started as soon as possible in patients with suspected A(H7N9) infection and suggest that corticosteroids should be avoided except in clinical situations where they have proven benefit. Although a prospective, randomized, controlled study is needed to definitely assess the clinical and virologic benefit of combination NAI therapy or higher dosing of oseltamivir, our findings in this observational study suggest that there is no benefit of higher oseltamivir dosing or use of multiple NAIs for treatment of A(H7N9)-infected patients. The increased frequency of emergence of resistance to NAIs during treatment and the high case-fatality proportion indicate an urgent need for improved antiviral treatment strategies, including drugs with different mechanisms of action than NAIs or those used in combination with NAIs, in hospitalized patients with A(H7N9) infection. Ideally, when available, new drugs should be evaluated in the context of randomized, controlled trials in A(H7N9)-infected patients.

Notes

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References

1. Gao R, Cao B, Hu Y, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med **2013**; 368:1888–97.
2. Wang X, Jiang H, Wu P, et al. Epidemiology of avian influenza A H7N9 virus in human beings across five epidemics in mainland China, 2013–17: an epidemiological study of laboratory-confirmed case series. Lancet Infect Dis **2017**; 17:822–32.
3. Zhou L, Ren R, Yang L, et al. Sudden increase in human infection with avian influenza A(H7N9) virus in China, September-December 2016. Western Pac Surveill Response J **2017**; 8:6–14.
4. Kile JC, Ren R, Liu L, et al. Update: increase in human infections with novel Asian lineage avian influenza A(H7N9) viruses during the fifth epidemic - China, October 1, 2016-August 7, 2017. MMWR Morb Mortal Wkly Rep **2017**; 66:928–32.
5. Kang M, Lau EHY, Guan W, et al. Epidemiology of human infections with highly pathogenic avian influenza A(H7N9)

virus in Guangdong, 2016 to 2017. Euro Surveill **2017**; 22. doi: 10.2807/1560-7917.ES.2017.22.27.30568.
6. Zhou L, Tan Y, Kang M, et al. Preliminary epidemiology of human infections with highly pathogenic avian influenza A(H7N9) virus, China, 2017. Emerg Infect Dis **2017**; 23:1355–9.
7. Uyeki TM, Katz JM, Jernigan DB. Novel influenza A viruses and pandemic threats. Lancet **2017**; 389:2172–4.
8. Short KR, Kroeze EJBV, Fouchier RAM, Kuiken T. Pathogenesis of influenza-induced acute respiratory distress syndrome. Lancet Infect Dis **2014**; 14:57–69.
9. Herold S, Becker C, Ridge KM, Budinger GR. Influenza virus-induced lung injury: pathogenesis and implications for treatment. Eur Respir J **2015**; 45:1463–78.
10. Lee N, Chan PK, Hui DS, et al. Viral loads and duration of viral shedding in adult patients hospitalized with influenza. J Infect Dis **2009**; 200:492–500.
11. McGeer A, Green KA, Plevneshi A, et al.; Toronto Invasive Bacterial Diseases Network. Antiviral therapy and outcomes of influenza requiring hospitalization in Ontario, Canada. Clin Infect Dis **2007**; 45:1568–75.
12. Kumar D, Michaels MG, Morris MI, et al.; American Society of Transplantation H1N1 Collaborative Study Group. Outcomes from pandemic influenza A H1N1 infection in recipients of solid-organ transplants: a multicentre cohort study. Lancet Infect Dis **2010**; 10:521–6.
13. Yu H, Liao Q, Yuan Y, et al. Effectiveness of oseltamivir on disease progression and viral RNA shedding in patients with mild pandemic 2009 influenza A H1N1: opportunistic retrospective study of medical charts in China. BMJ **2010**; 341:c4779.
14. Louie JK, Yang S, Acosta M, et al. Treatment with neuraminidase inhibitors for critically ill patients with influenza A (H1N1)pdm09. Clin Infect Dis **2012**; 55:1198–204.
15. Muthuri SG, Myles PR, Venkatesan S, Leonardi-Bee J, Nguyen-Van-Tam JS. Impact of neuraminidase inhibitor treatment on outcomes of public health importance during the 2009–2010 influenza A(H1N1) pandemic: a systematic review and meta-analysis in hospitalized patients. J Infect Dis **2013**; 207:553–63.
16. Muthuri SG, Venkatesan S, Myles PR, et al.; PRIDE Consortium Investigators. Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted to hospital with influenza A H1N1pdm09 virus infection: a meta-analysis of individual participant data. Lancet Respir Med **2014**; 2:395–404.
17. Chan PK, Lee N, Zaman M, et al. Determinants of antiviral effectiveness in influenza virus A subtype H5N1. J Infect Dis **2012**; 206:1359–66.
18. Fielding JE, Kelly HA, Mercer GN, Glass K. Systematic review of influenza A(H1N1)pdm09 virus shedding: duration is affected by severity, but not age. Influenza Other Respir Viruses **2014**; 8:142–50.
19. Zheng S, Wang Y, Yu F, et al. Benefit of early initiation of neuraminidase inhibitor treatment to hospitalized patients with avian influenza A (H7N9) virus. Clin Infect Dis **2017**. doi: 10.1093/cid/cix930.
20. Cao B, Gao H, Zhou B, et al. Adjuvant Corticosteroid Treatment in Adults With Influenza A (H7N9) Viral Pneumonia. Crit Care Med **2016**; 44:e318–28.
21. Gao HN, Lu HZ, Cao B, et al. Clinical findings in 111 cases of influenza A (H7N9) virus infection. N Engl J Med **2013**; 368:2277–85.
22. Tang BM, Craig JC, Eslick GD, Seppelt I, McLean AS. Use of corticosteroids in acute lung injury and acute respiratory distress syndrome: a systematic review and meta-analysis. Crit Care Med **2009**; 37:1594–603.
23. Arabi YM, Mandourah Y, Al-Hameed F, et al. Corticosteroid therapy for critically ill patients with the middle east respiratory syndrome. Am J Respir Crit Care Med **2017**. doi: 10.1164/rccm.201706-1172OC.
24. de Jong MD, Simmons CP, Thanh TT, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med **2006**; 12:1203–7.
25. Gu J, Xie Z, Gao Z, et al. H5N1 infection of the respiratory tract and beyond: a molecular pathology study. Lancet **2007**; 370:1137–45.
26. Hui DS, Lee N, Chan PK, Beigel JH. The role of adjuvant immunomodulatory agents for treatment of severe influenza. Antiviral Res **2018**; 150:202–16.
27. Lee N, Allen Chan KC, Hui DS, et al. Effects of early corticosteroid treatment on plasma SARS-associated Coronavirus RNA concentrations in adult patients. J Clin Virol **2004**; 31:304–9.
28. Hu Y, Lu S, Song Z, et al. Association between adverse clinical outcome in human disease caused by novel influenza A H7N9 virus and sustained viral shedding and emergence of antiviral resistance. Lancet **2013**; 381:2273–9.
29. Ke C, Mok CKP, Zhu W, et al. Human infection with highly pathogenic avian influenza A(H7N9) virus, China. Emerg Infect Dis **2017**; 23:1332–40.
30. Duval X, van der Werf S, Blanchon T, et al.; Bivir Study Group. Efficacy of oseltamivir-zanamivir combination compared to each monotherapy for seasonal influenza: a randomized placebo-controlled trial. PLoS Med **2010**; 7:e1000362.
31. South East Asia Infectious Disease Clinical Research N. Effect of double dose oseltamivir on clinical and virological outcomes in children and adults admitted to hospital with severe influenza: double blind randomised controlled trial. BMJ **2013**; 346:f3039.

32. Adisasmito W, Chan PK, Lee N, et al. Effectiveness of antiviral treatment in human influenza A(H5N1) infections: analysis of a Global Patient Registry. J Infect Dis **2010**; 202:1154–60.
33. Yang SG, Cao B, Liang LR, et al.; National Influenza A Pandemic (H1N1) 2009 Clinical Investigation Group of China. Antiviral therapy and outcomes of patients with pneumonia caused by influenza A pandemic (H1N1) virus. PLoS One **2012**; 7:e29652.
34. Lee N, Hui DS, Zuo Z, et al. A prospective intervention study on higher-dose oseltamivir treatment in adults hospitalized with influenza a and B infections. Clin Infect Dis **2013**; 57:1511–9.
35. Widmer N, Meylan P, Ivanyuk A, Aouri M, Decosterd LA, Buclin T. Oseltamivir in seasonal, avian H5N1 and pandemic 2009 A/H1N1 influenza: pharmacokinetic and pharmacodynamic characteristics. Clin Pharmacokinet **2010**; 49:741–65.
36. Bantia S, Parker CD, Ananth SL, et al. Comparison of the anti-influenza virus activity of RWJ-270201 with those of oseltamivir and zanamivir. Antimicrob Agents Chemother **2001**; 45:1162–7.
37. Davies BE. Pharmacokinetics of oseltamivir: an oral antiviral for the treatment and prophylaxis of influenza in diverse populations. J Antimicrob Chemother **2010**; 65(Suppl 2):ii5–10.
38. Zhang Y, Gao H, Liang W, et al. Efficacy of oseltamivir-peramivir combination therapy compared to oseltamivir monotherapy for Influenza A (H7N9) infection: a retrospective study. BMC Infect Dis **2016**; 16:76.
39. Dunning J, Baillie JK, Cao B, Hayden FG; International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC). Antiviral combinations for severe influenza. Lancet Infect Dis **2014**; 14:1259–70.