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Comparison between human infections caused by highly and low pathogenic H7N9 avian influenza viruses in Wave Five: Clinical and virological findings

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SUMMARY

Objective: The newly emerged highly pathogenic (HP) H7N9 avian influenza virus during Wave Five has caused 28 human infections, while differences in disease severity between low pathogenic (LP)- and HP-H7N9 human infections remain unclear.

Methods: Clinical data, concentrations of serum cytokines, dynamics of virus shedding and PaO_2/FiO_2 from patients infected with LP-H7N9 ($n=7$, LP group) and HP-H7N9 ($n=5$, HP group) viruses during Wave Five were compared. In addition, critical mutations associated with H7N9 virulence in mammal/human were analyzed.

Results: Lymphopenia, elevated aspartate aminotransferase, alanine aminotransferase, C-reactive protein and lactate dehydrogenase were common features, with higher incidences of leukopenia and thrombocytopenia in the LP group. The acute phase of both groups was accompanied with elevated cytokines associated with disease severity, including MIF, MCP-1 and IP-10. Diffuse exudation of the lungs and consolidation were observed from all patients. The dynamics of virus shedding and PaO_2/FiO_2 were similar between both groups. Notably, a higher prevalence of neuraminidase inhibitors (NAIs) resistance in the HP-H7N9 virus was found.

Conclusions: Our results indicate that this newly emerged HP-H7N9 virus caused similar disease severity in humans compared with LP-H7N9 virus, while higher case fatality rate and prevalence of NAI-resistance in human HP-H7N9 infections were of great concern.

Introduction

Human infections with H7N9 avian influenza virus (AIV) were first reported in 2013,[1] and there have been five waves of H7N9 virus in human beings thus far in China. During the first four waves, a total of 798 human infections with a case fatality rate (CFR) of 40.6% (324 deaths) were reported.[2] The fifth wave, which started in October 2016, had 766 laboratory-confirmed cases with

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288 deaths (CFR: ~37.6%) from mainland China, Taiwan, Hong Kong, and Macau as of September, 2017, almost as many as the first four waves combined.[2] During the fifth wave, H7N9 viruses were reported in 31 provinces/regions/municipalities in China, and detected for the first time in Western China including Gansu, Shaanxi, Sichuan, Chongqing and Tibet, indicating a geographical spread.[3,4] Consistent with previous outbreaks,[5] the H7N9 viruses in the fifth wave genetically derived from the Pearl River Delta lineage or the Yangtze River Delta lineage.

During the first four waves, circulating H7N9 viruses among poultry and humans in China were classified as low pathogenic (LP) AIV, which cause asymptomatic infection in poultry.[6] However, the Chinese Center for Disease Control and Prevention (China CDC) reported the identification of a new H7N9 variant from two patients in Guangdong Province in the fifth wave.[4,7] These new isolates contained a cleavage site (CS) with polybasic amino acids (AAs) by insertion of four AAs ("KRTA", "KRIA" or "KRAA") into the HA protein,[3,7] and become highly pathogenic (HP) to chickens.[8–10] Human infections with HP-H7N9 virus were significantly more likely to occur in rural areas, and exposure to sick or dead poultry was the most important risk factor.[10,11] Overall, there have been 28 case reports of HP-H7N9 infections from Taiwan, Guangxi, Guangdong, Hunan, Shaanxi, Hebei, Henan, Fujian and Yunnan provinces.[12] Meanwhile, HP-H7N9 virus has caused several outbreaks in poultry farms, resulting in the deaths of approximately 110,000 poultry over 10 provinces.[13] Recent studies have found that some HP-H7N9 strains were more pathogenic than LP-H7N9 virus in mammals and able to transmit among ferrets via respiratory droplets, posing an increased threat to public health.[14–16]

The fifth wave of human H7N9 infections differed from past outbreaks in that it is driven by both HP and LP variants,[3] but it is unknown whether there are any differences in disease severity between humans infected with LP- or HP-H7N9 viruses. Here, we described and directly compared clinical and virological factors associated with disease severity between LP- and HP-H7N9 human infections during Wave Five.

Patients and methods

Data collection and analysis of clinical findings

Clinical information, including complete blood counts, blood biochemistry, chest radiographs and computed tomographic (CT) scans from ten patients with confirmed infection of H7N9 virus were collected at the earliest time-point upon hospitalization and afterwards, among whom two patients were from Yunnan Province (cases YN01 and YN02) and the rest were from Shenzhen municipality of Guangdong Province (cases SZ01-SZ08). These results were combined with data from two previously published HP-H7N9 cases (designated GZ01 and GZ02).[7,17] In addition, healthy controls ($N=6$) were also included. The study was performed in accordance with guidelines approved by the Ethics Committees from Shenzhen Third People's Hospital (SZTHEC2016001) and Yunnan Center for Disease Control and Prevention Ethics Committee (YNCDC2017001), and verbal informed consents were obtained from all patients or patients' family members.

Quantitative reverse transcription polymerase chain reaction

The viral load was measured on sputum from the respiratory tract collected from patients at various time-points after hospitalization. Viral RNAs were extracted from samples using the QIAamp RNA Viral Kit (Qiagen, Heiden, Germany) and quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using an in-house assay as previously described[18] and a commercial kit for H7N9 virus detection (Mabsky Biotech Co., Ltd., Shenzhen, China). The specimens were considered positive if Ct value is ≤38.0, while negative if the results were undetermined. Specimens with a Ct higher than 38 were repeated. The specimen was considered positive if the repeat results were the same as before and between 38–40. If the repeat Ct was undetectable, the specimen was considered negative.

Cytokine and chemokine measurements

Cytokines and chemokines were measured from patient sera collected at the earliest time-point after hospitalization using the RayBio® Human Cytokine Antibody Array 5 (GSeries, Product Code: AAH-CYT-G5, RayBiotech Inc., Norcross, USA) and the LuxScan 10 K Microarray Scanner (Captial Bio, Beijing, China) following manufacturer instructions.

Quantification of hypoxia and lung injury

The partial pressure of oxygen (PaO_2) in arterial blood taken from the patients at various time–points after hospitalization was measured by the ABL90 blood gas analyzer (Radiometer). The fraction of inspired oxygen (FiO_2) is calculated by the following formula: $FiO_2 = (21 + \text{oxygen flow (in units of l/min)} * 4) / 100$. The PaO_2/FiO_2 ratio (in units of mmHg) is calculated by dividing the PaO_2 value with the FiO_2 value. A PaO_2/FiO_2 ratio less than or equal to 100 mmHg is considered one of the criteria for severe acute respiratory distress syndrome (ARDS).

Statistical analysis

Fisher exact test or χ^2 test was used for comparing the proportions. The unpaired, two-tailed *t*-test was used to determine whether differences in the cytokine levels were statistically significant. A *p*-value between 0.01–0.05, 0.001–0.01 and below 0.001 was considered statistically significant (*), very significant (**) and extremely significant (***), respectively. Statistical analysis was performed using GraphPad Prism.

Results

Timeline and general features of LP- and HP-H7N9 infection in humans

Twelve human infections with LP-H7N9 ($n=7$, LP group) and HP-H7N9 ($n=5$, HP group) viruses during Wave Five were included in our study group (Fig. 1). Genetic characterization of LP- or HP-H7N9 viruses was carried out in retrospect, thus patients in both groups were managed in the same manner. Patients YN01 and YN02 were mother and daughter, while the rest were not epidemiologically linked. Most of the patients (66.7%) were found to have prior exposure to poultry or live poultry markets (LPMs), with no difference between the LP and HP groups (Fig. 1). All the HP-H7N9 virus infected patients who had exposure history contacted with sick or dead chickens, which was consistent with the enhanced virulence of HP-H7N9 virus in chickens. The time from illness onset to hospital admission as well as illness onset to laboratory confirmation were similar for both groups. All the patients were admitted to hospital between 3–10 days post illness onset. Most of the patients (11/12, 91.7%) developed ARDS and mechanical ventilation was used. Additionally, analysis of the epidemiological characteristics showed that the median age, interval from onset-admission days, co-existing medical conditions and incidence of complications during hospitalization of HP-H7N9 human infections were similar with LP-H7N9 human infections in Wave Five, and also LP-H7N9 human infections in first four waves (Table S1).

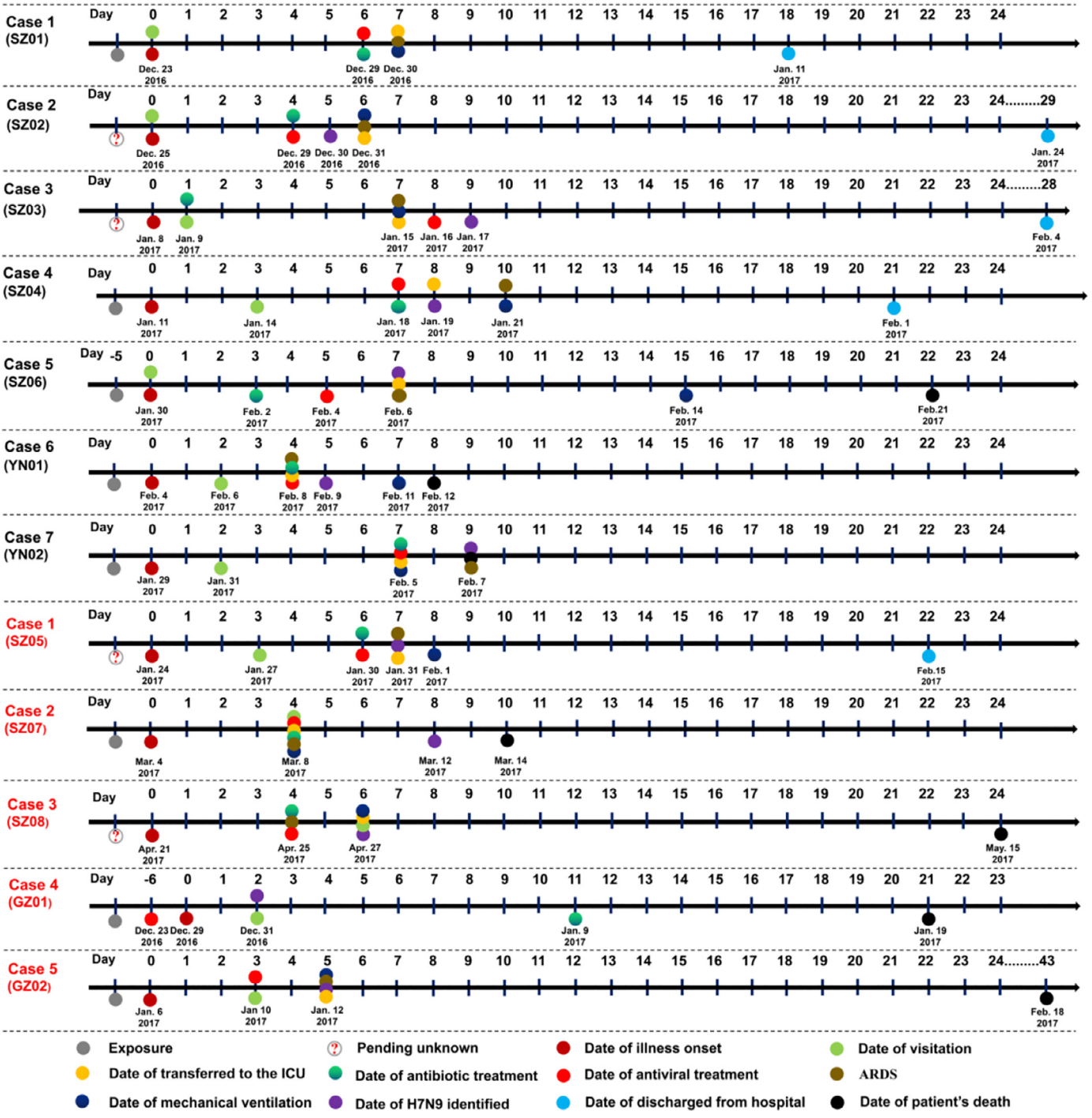

Fig. 1. A timeline of human infections with LP- and HP-H7N9 viruses. Patients were designated according to the geographical location and chronological order based on disease report date. Cases numbers in black represent LP-H7N9 virus infected cases, while cases numbers in red represent HP-H7N9 virus infected cases. Various milestones in the disease course were displayed with different-colored circles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Clinical comparison of LP- and HP-H7N9 infection in humans

Patients infected with both LP- and HP-H7N9 viruses initially showed flu-like symptoms including fever, headache, cough and chills, and developed into shortness of breath quickly. In moribund patients, the common outcomes were ARDS, severe pneumonia, multiple organ failure and death between 8-43 days after symptom onset (d.a.o.) (Fig. 1, Table S1). The case fatality rate of the HP group (14/28, 50%) was slightly higher than that of LP-H7N9 human infections from the first four waves (324/798, 40.6%), as well as Wave Five (288/766, 37.6%).[12,19] A complete blood count was measured for each patient either on the date of hospital admission, or at the earliest time-point thereafter (Table 1). Lymphopenia, elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT), C-reactive protein (CRP) and lactate dehydrogenase (LDH) were common features of both groups, while the incidence rate of leukopenia and thrombocytopenia was higher in the LP group (Table 1).[7,17,20]

The CT scans showed large pieces of consolidation, the fusion trend between lobe lesions, ground-glass opacity around the

Table 1
Clinical Characteristics and laboratory results on subjects hospitalized with infection of LP and HP-H7N9 during the five waves in China.

Parameter[a]	HP (Wave Five, $N=5$)[b,c]	LP (Wave Five, $N=7$)	*P* value	LP (First four waves, $n=123$)[d]	*P* value[e]
WBC ($\times10^9$/L)[+]	5.02 (4.81–5.6)	3.88 (3.52–6.39)	NA	4.5 (2.9–6.2)	NA
LYM ($\times10^9$/L) [+]	0.505 (0.48–0.56)	0.53 (0.47–0.86)	NA	0.5 (0.3–0.7)	NA
NEU ($\times10^9$/L)[+]	4.18 (4.13–4.73)	3.22 (3.1–4.97)	NA	3.3 (2.2–5.4)	NA
PLT ($\times10^9$/L)[+]	166 (160–221.5)	157 (139–198.3)	NA	114 (82–147.5)	NA
AST (U/L)[+]	64.2 (38.5–107.4)	88.3 (42.85–224.9)	NA	53 (38–96.5)	NA
ALT (U/L)[+]	63.7 (40.4–112.4)	74 (44.05–119.7)	NA	35.5 (24–64.5)	NA
CRE (μmol/L)[+]	60 (53–107.15)	89.65 (62.1–106.6)	NA	70.7 (58.3–85)	NA
CK (U/L)[+]	182 (144–239.5)	163.8 (123.75–354)	NA	195 (96–562)	NA
CRP (nmol/L)[+]	92.65 (74.6–176.2)	72.5 (49.7–83.3)	NA	65 (25–113)	NA
ALB (g/L)[+]	31.9 (26.3–32.5)	32.6 (32.5–33.7)	NA	NA	NA
LDH (U/L)[+]	711.5 (573.8–848.5)	994.5 (475.8–1596.5)	NA	498 (388–661)	NA
Leukopenia	0/4 (0%)	4/7 (57%)	0.194	48/105 (46%)	0.129
Lymphopenia	4/4 (100%)	5/5 (100%)	NA	88/99 (89%)	1.000
Neutropenia	0/4 (0%)	0/6 (0%)	NA	13/103 (13%)	1.000
Neutrophilia	0/4 (0%)	0/6 (0%)	NA	5/103 (5%)	1.000
Thrombocytopenia	0/3 (0%)	3/6 (50%)	0.464	80/104 (77%)	0.015
Elevated AST	2/4 (50%)	4/7 (57%)	1.000	54/103 (52%)	1.000
Elevated ALT	2/3 (66.7%)	4/6 (66.7%)	1.000	34/100 (34%)	0.279
Elevated CRE	1/3 (33.3%)	1/6 (16.7%)	1.000	11/103 (11%)	0.305
Elevated CK	2/4 (50%)	2/6 (33.3%)	1.000	48/98 (49%)	1.000
Elevated CRP	4/4 (100%)	5/5 (100%)	NA	83/92 (90%)	1.000
Elevated LDH	4/4 (100%)	6/6 (100%)	NA	89/98 (91%)	1.000

NA: Not applicable.
Abbreviations: WBC: white blood cell; LYM: lymphocyte; NEU: neutrophil; PLT: platelet; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRE: serum creatinine; CK: creatine kinase; CRP: C-reactive protein; LDH: lactate dehydrogenase.
Age specific reference ranges used to define abnormalities in blood results: Leukopenia ($\times10^9$/L): 2 months–2 years: <5, >2 years: <4; Lymphopenia ($\times10^9$/L): 2–11 months: <4.0, 1–11 years: <1.5, 12+ years: <1; Neutropenia ($\times10^9$/L): All ages: <1.5; Neutrophilia ($\times10^9$/L): All ages: >8.5; Thrombocytopenia ($\times10^9$/L): All ages: <150; Elevated AST (U/L): All ages: >50; Elevated ALT (U/L): All ages: >50; Elevated CRE (μmol/L): All ages: >120; Elevated CK (U/L): All ages: >200; Elevated CRP (nmol/L): All ages: >10; Elevated LDH (U/L): <7 years: >400, 7–15 years: >300, 16+ years: >250.
[a] Results were obtained from patients at the earliest available time-point after hospitalization.
[b] Clinical data from two HP-H7N9 human cases were presented in this study. In addition, three cases from previous reports were included in this analysis.[7,17]
[c] Reference group.
[d] Clinical data of the 123 cases from previous report was included in this analysis.[20]
[e] A *p*-value between 0.01–0.05, 0.001–0.01 and below 0.001 was considered statistically significant, very significant and extremely significant, respectively.
[+] Values shown represent the mean and inter-quartile range (IQR).

shadow, unclear lung structure and pleural effusion were common features of the patients in both groups (Fig. 2). In addition, bilateral pulmonary portal shadow increased and thickened as the progression of disease. Chest radiographs of patients SZ02, SZ03, SZ06 (LP group), as well as SZ05 and SZ08 (HP group) at different days after admission (d.a.a.) were also shown (Figure S1). Patchy shadows were diffusely distributed in the lungs. Consistent with the CT scan, the chest radiographs showed diffuse exudation of the lungs and consolidation.

Cytokines associated with disease severity

Innate and adaptive immune responses, coordinated by cytokines, are activated shortly after infection to provide protection. Previous studies have found that several pro-inflammatory cytokines are associated with the disease severity of H7N9 infection, including IFN-γ, IL-8, IL-16, MCP-1, MIG, SCF, HGF, IP-10 and MIF.[21–23] Of the nine cytokines, MIF, MCP-1 and IP-10 were elevated significantly in both LP- and HP groups compared with healthy controls, while there were no statistical differences between the LP and HP groups (Fig. 3). For the other six cytokines, the three groups showed similar expression levels, with the exception of IFN-γ (only HP group is higher than the healthy control, while comparable to LP group) (Fig. 3).

Viral loads and lung function during treatment

We continuously monitored the viral loads of H7N9 virus in the sputum of the patients by qRT-PCR upon admission and during subsequent antiviral treatment (Fig. 4). Firstly, we plotted the viral load of the first available sputum taken after admission for each patient in relation to the duration of illness (Fig. 4A), and the median Ct values of the LP and HP groups were 29.16 and 25.1, respectively. The time from illness onset to initiation of antiviral treatment was between 3–8 days in both groups, except patient GZ01 in the HP group who received prophylactic antiviral drugs before illness onset (Fig. 1). During antiviral treatment with NAIs, the viral loads in most patients of both groups decreased rapidly and became negative within eight days, except patient SZ02 in the LP group (Fig. 4B).

As an indicator of the severity of ARDS, dynamic changes of PaO_2/FiO_2 during the treatment were shown (Fig. 4C). Patients SZ01, SZ02, SZ06 in the LP group, and SZ05, SZ08 in the HP group developed severe ARDS upon admission. During the treatment, the PaO_2/FiO_2 ratio of these patients increased significantly except patient SZ07 in the HP group, who developed severe ARDS during the treatment.

Viral factors associated with virulence

We investigated key mutations that has been shown to significantly affect the biologic properties of the viruses in mammals (Table 2).[15,24–27] Most of the H7N9 isolates were from samples collected shortly after the initiation of antiviral treatment, except patient GZ01 in the HP group (12 days after antiviral treatment). A mutation contributed to NAI-resistance (R292K (N2 numbering) substitution in NA), which has been reported to be associated with adverse clinical outcomes,[28] was identified in the NA gene of the viruses isolated from patients SZ07, SZ08, GZ01, GZ02 in the HP group, while only patient SZ04 in the LP group. The E627K and D701N mutation in the PB2 segment of H7N9 virus were shown to be responsible for the enhanced virulence and transmission in mice and ferrets, and K526R mutation was associated with the en-

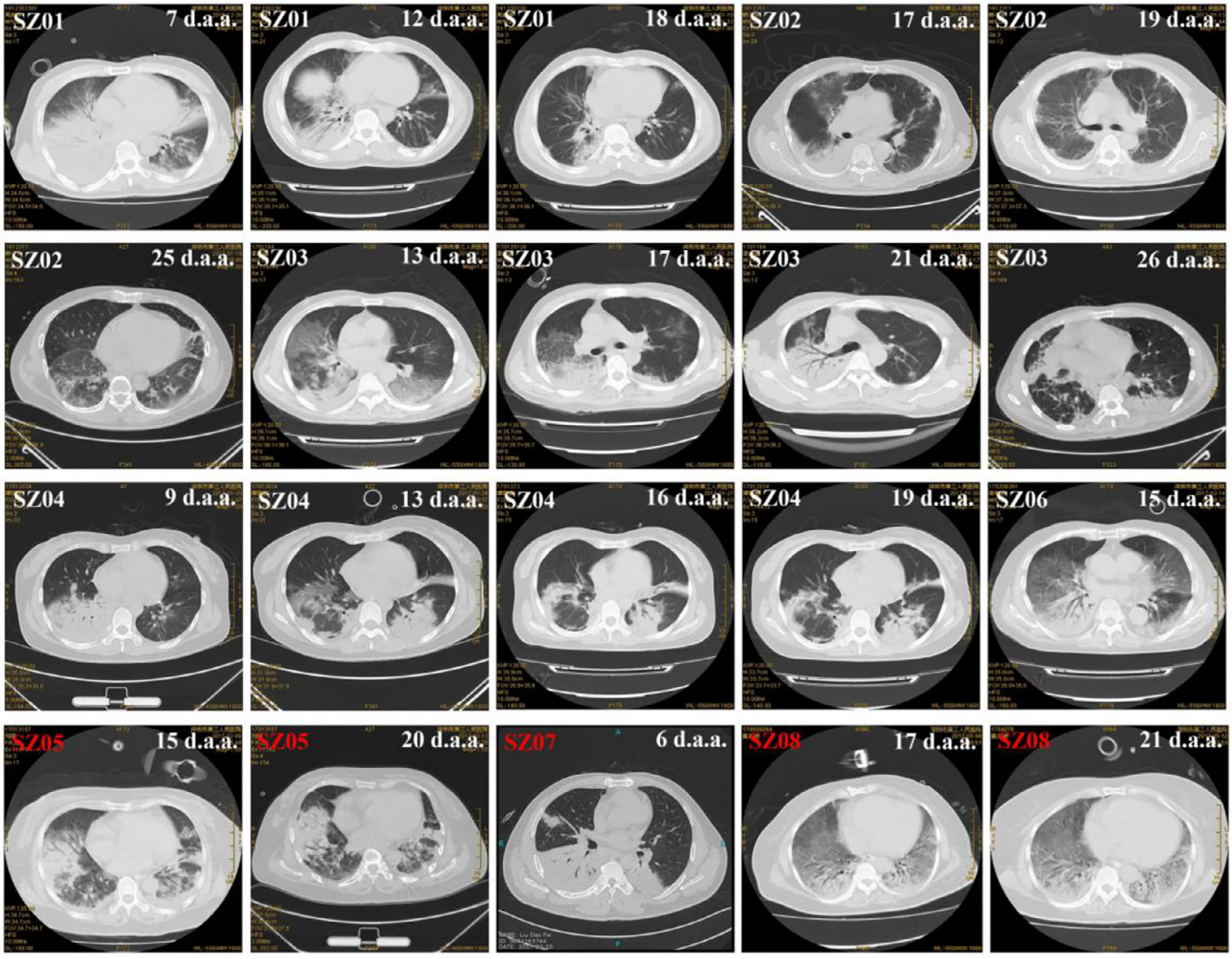

Fig. 2. Computed tomographic (CT) scans of H7N9 patients. CT scans of patients SZ01-SZ08 taken at indicated days after admission (d.a.a.) were shown, and case numbers in red represent the HP-H7N9 virus infected cases. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Molecular characteristics of the HA, NA and PB2 genes of the H7N9 viruses isolated from patients in this study.

Patient No.	Virus isolates	Sample collection date (days post antiviral treatment)	Key molecular changes				
			HA cleavage site	NA R292K	K526R	PB2 E627K	D701N
SZ01	A/Shenzhen/Th001/2016(H7N9)	2	PEIPKGR/GL	R	K	K	D
SZ02	A/Shenzhen/Th002/2016(H7N9)	1	PEIPKGR/GL	R	K	K	D
SZ03	A/Shenzhen/Th003/2017(H7N9)	0	PEIPKGR/GL	R	K	E	D
SZ04	A/Shenzhen/Th004/2017(H7N9)	4	PEIPKGR/GL	K	K	E	D
SZ06	A/Shenzhen/Th006/2017(H7N9)	3	PEIPKGR/GL	R	K	K	D
YN01	A/Yunnan/YN001/2017(H7N9)	1	PEIPKGR/GL	R	K	K	D
YN02	A/Yunnan/YN002/2017(H7N9)	1	PEIPKGR/GL	R	K	K	D
SZ05	A/Guangdong/Th005/2017(H7N9)	1	PEVPKGKRIAR/GL	R	R	K	D
SZ07	A/Shenzhen/Th007/2017(H7N9)	5	PEVPKRKRTAR/GL	K	K	K	D
SZ08	A/Shenzhen/Th008/2017(H7N9)	2	PEVPKRKRTAR/GL	K	R	E	N
GZ01	A/Guangdong/Th008/2017(H7N9)	12	PEVPKRKRTAR/GL	K	K	E	D
GZ02	A/Guangdong/17SF006/2017(H7N9)	2	PEVPKRKRTAR/GL	K	K	K	D

hancement K627 and N701 function.[15,24,25,29,30] Most of the isolates were found to possess the E627K mutation, except the isolates from patients SZ03, SZ04 in the LP group, and patients SZ08, GZ01 in the HP group. Notablely, the D701N and K526R mutations were only found in the isolate from the HP group, SZ08 for D701N, SZ05 and SZ08 for K526R.

Discussion

In this study, we investigated and compared the clinical and virological factors associated with disease severity between LP- and HP-H7N9 human infections during Wave Five. Consistent with previous reports,[4,9,31,32] there were no significant differences between

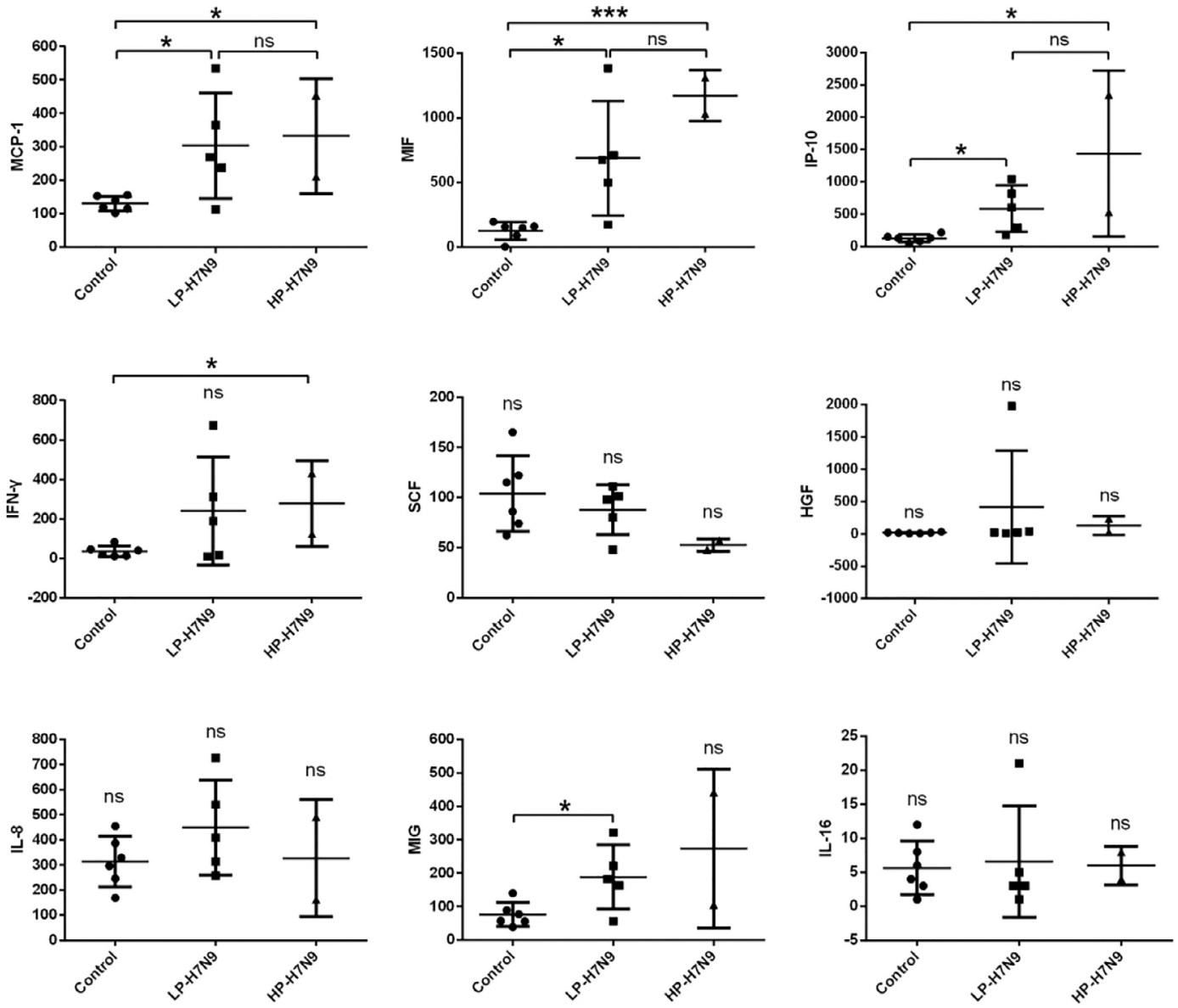

Fig. 3. Comparison of serum cytokines between LP- and HP-H7N9 viruses infected patients. Samples from patients infected with LP- and HP-H7N9 viruses were collected at the earliest possible time-point after hospitalization. Healthy subjects were involved as control. Values were presented in units of relative abundance. The mean +/- standard error was shown for each group and the *p*-values (as determined by *t*-test) above the various groups represented the comparison of means between two groups. A *p*-value between 0.01–0.05, 0.001–0.01 and below 0.001 was considered statistically significant (*), very significant (**) and extremely significant (***), respectively, whereas ns represents not significant.

the HP-H7N9 and LP-H7N9 human infections in first four waves and Wave Five in age, days from onset to hospital admission, co-existing chronic medical conditions and incidence of complications during hospitalization (Table S1). The majority of the enrolled LP- and HP-H7N9 virus infected cases showed severe respiratory disease, in which hydrothorax, hypercytokinemia and ARDS, in addition to abnormalities in complete blood counts and serum biochemistry, were readily observed (Table 1 and Figs. 3–4). The acute phase of LP- and HP-H7N9 infection was accompanied with elevated cytokines (e,g, MIF, MCP-1 and IP-10), and both groups had a similar profile (Fig. 3). Imageology characteristics of these H7N9 patients after admission (Fig. 2 and S1) suggest serious pulmonary disease associated with H7N9 infection and support the diagnosis of ARDS. While there were no significant differences of imageology characteristics associated with pathological changes between the two groups. In addition, patients in both groups showed similar pattern of PaO_2/FiO_2 dynamics. These data indicated that the LP- and HP-H7N9 viruses cause similar disease severity in humans.

Although the patients were admitted at varying time-points post illness onset, the time of antiviral treatment did not exceed two days before admission. The viral loads in the sputum, collected soon after admission from the patient groups, taken as a whole could provide an aggregate profile over the course of illness before therapeutic intervention.[28] According to the results of qRT-PCR, it appeared that the viral loads in the HP group were similar with the LP group (Fig. 4). This is not consistent with a previous study, which showed that HP-H7N9 virus replicated to a higher level than the LP-H7N9 virus in ferrets.[16] While due to the limited cases of enrolled cases, this difference needs further confirmation. All the available sequences of human-derived HP-H7N9 viruses were downloaded from Global Initiative on Sharing All Influenza Data (GISAID) database for further analysis. The presence of K627 (17/45, 37.8%), N701 (6/45, 13.3%) and R526 (16/45, 35.6%) in PB2

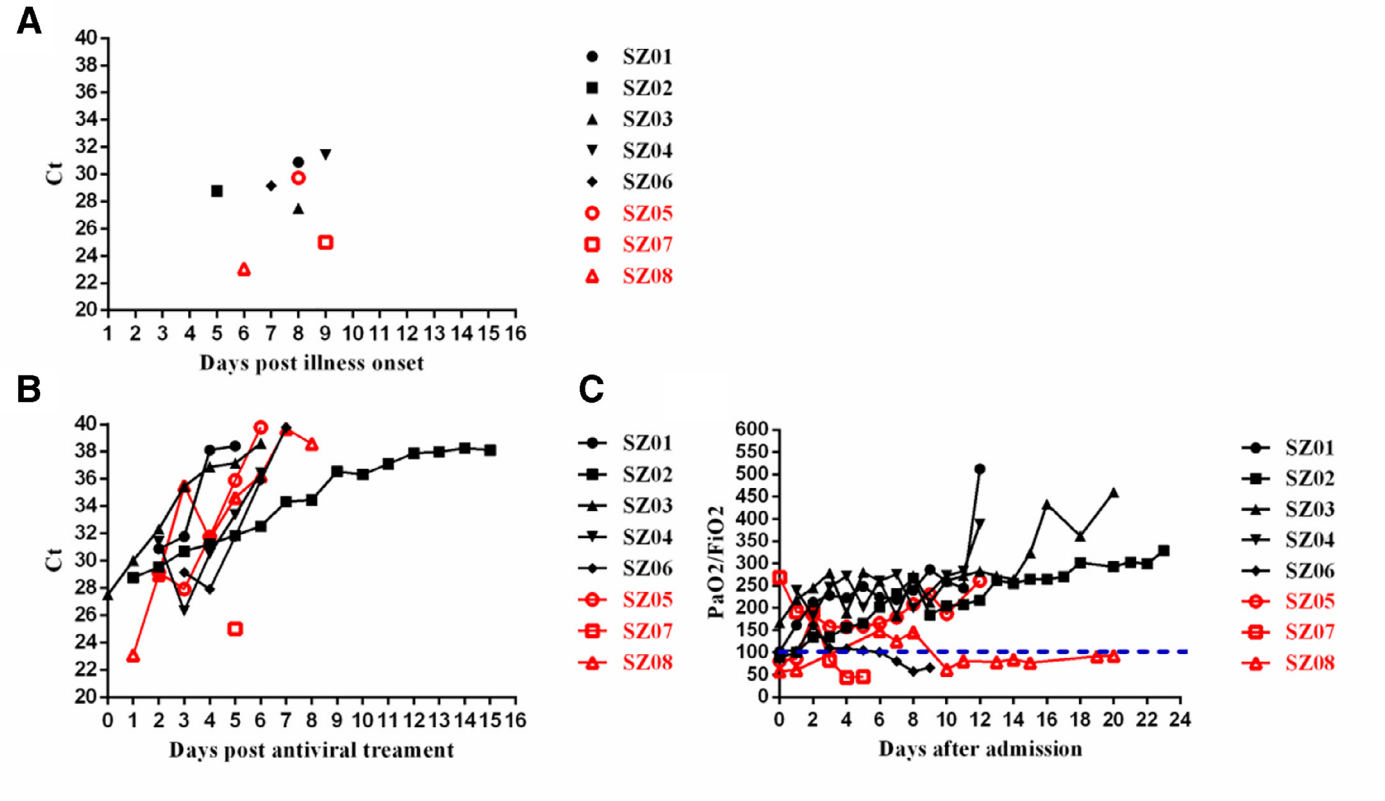

Fig. 4. Dynamic changes of viral loads and PaO_2/FiO_2 ratio of H7N9 virus infected patients during antiviral treatment. A and B. Viral loads in sputum of the earliest possible time-point upon hospitalization and serial viral loads after antiviral treatment were shown as Ct values. C. Serial PaO_2/FiO_2 ratio during hospitalization. Values from patients with HP-H7N9 virus were in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of the available HP-H7N9 viruses suggested that the virulence and transmission of viruses might be enhanced in human, which is of concern. A recent study has also found that R482, V588 in the PB2 segment, and R497 in the PA segment contributed to the enhanced polymerase activity of HP-H7N9 virus.[27] Analysis of all the available HP-H7N9 viruses showed that PB2- R482, PB2-V588 and PA-R497 are present in 13.3% (6/45), 35.6% (16/45) and 13.3% (6/45), respectively (Table S2). Importantly, 13.3% of the HP-H7N9 viruses possessed all the three mutations. The presence of these mutations further potentiates the public threat of this virus.

A previous study has found that sustained viral shedding and emergence of antiviral resistance is associated with adverse clinical outcomes for H7N9 human infections.[28] The R292K mutation in NA was found in most of the patients in the HP group (4 out of 5), while only patient SZ04 (1 out of 7) in the LP group. To confirm this trend, we further analyzed the NAI-resistance mutation in all available sequences of HP-H7N9 virus from humans. The overall incidence rate of NAI-resistance in HP-H7N9 virus is about 28% (13/46) (Table S2), which is far higher than that of LP-H7N9 virus (34/1225, 2.8%). This suggests that HP-H7N9 virus may be easier to develop NAI-resistance mutation during the antiviral treatment with NAIs, which should be closely monitored during treatment of HP-H7N9 virus infected cases, such as using the recently developed qRT-PCR assay to simultaneously detect the presence of HP-H7N9 virus and monitor the occurrence of NAI-resistance mutation.[18]

The emergence of HP-H7N9 virus and infections in humans are a comparatively recent finding compared to the human infections with LP-H7N9 virus, and for the first time we directly compared the clinical and virological factors associated with disease severity between LP- and HP-H7N9 human infections during Wave Five. Although there was only a small number of HP-H7N9 virus infected cases, our results, together with previous epidemiological studies, indicated that this newly emerged HP-H7N9 variant caused similar disease severity in humans when compared to LP-H7N9 virus. The higher frequency of NAI-resistance mutation in HP-H7N9 virus, which might be associated with the higher case fatality rate, needs to be closely monitored and considered during the antiviral treatment.

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Conflicts of interest

The authors have declared that no conflicts of interest exist.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.01.005.

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