



Association between adverse clinical outcome in human disease caused by novel influenza A H7N9 virus and sustained viral shedding and emergence of antiviral resistance

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Summary

Background On March 30, a novel influenza A subtype H7N9 virus (A/H7N9) was detected in patients with severe respiratory disease in eastern China. Virological factors associated with a poor clinical outcome for this virus remain unclear. We quantified the viral load and analysed antiviral resistance mutations in specimens from patients with A/H7N9.

Methods We studied 14 patients with A/H7N9 disease admitted to the Shanghai Public Health Clinical Centre (SPHCC), China, between April 4, and April 20, 2013, who were given antiviral treatment (oseltamivir or peramivir) for less than 2 days before admission. We investigated the viral load in throat, stool, serum, and urine specimens obtained sequentially from these patients. We also sequenced viral RNA from these specimens to study the mutations associated with resistance to neuraminidase inhibitors and their association with disease outcome.

Findings All patients developed pneumonia, seven of them required mechanical ventilation, and three of them further deteriorated to become dependent on extracorporeal membrane oxygenation (ECMO), two of whom died. Antiviral treatment was associated with a reduction of viral load in throat swab specimens in 11 surviving patients. Three patients with persistently high viral load in the throat in spite of antiviral therapy became ECMO dependent. An Arg292Lys mutation in the virus neuraminidase (NA) gene known to confer resistance to both zanamivir and oseltamivir was identified in two of these patients, both also received corticosteroid treatment. In one of them, wild-type sequence Arg292 was noted 2 days after start of antiviral treatment, and the resistant mutant Lys292 dominated 9 days after start of treatment.

Interpretation Reduction of viral load following antiviral treatment correlated with improved outcome. Emergence of NA Arg292Lys mutation in two patients who also received corticosteroid treatment led to treatment failure and a poor clinical outcome. The emergence of antiviral resistance in A/H7N9 viruses, especially in patients receiving corticosteroid therapy, is concerning, needs to be closely monitored, and considered in pandemic preparedness planning.

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Introduction

Pandemic influenza arises from influenza viruses of birds or swine.¹ Recently, we and others reported a novel avian-origin influenza virus subtype (A/H7N9) from patients with severe and fatal respiratory disease in eastern China.^{2,3} The virus has so far caused disease in 132 human beings in nine provinces and municipalities in China, leading to 33 deaths. Poultry seem to be the source of human infections.³ Although other avian influenza viruses (eg, subtypes H5N1, H9N2, H7N7, and H7N3) have infected man before, such transmission has remained exceedingly inefficient, possibly because avian viruses are inefficient at binding to the sialic acid receptors located in the human upper airways. By comparison, the novel A/H7N9 virus, which has mammalian adaptation mutations in the receptor binding site of the haemagglutinin gene and in the

polymerase basic 2 (PB2) gene (E627K)^{2,4} of the virus, seems to cross species from poultry to man more easily, raising concern about its pandemic potential.

The genes of this A/H7N9 virus were of avian origin, with six internal genes from avian influenza A (H9N2) viruses whereas the haemagglutinin (HA) and neuraminidase (NA) gene segments derive from viruses from ducks or wild birds.^{2,3} Human beings infected with A/H7N9 are of older age and many of them have underlying diseases.⁵ Since A/H7N9 is resistant to the M2-ion channel blockers amantadine and rimantadine, the neuraminidase inhibitors oseltamivir, zanamivir, and peramivir have been the main drugs used for antiviral treatment of patients with A/H7N9.

The viral and host factors associated with the unusual severity of this disease are poorly understood. In this study, we quantified the viral load in sequentially obtained

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throat swabs, serum, urine, and stool specimens from A/H7N9 patients. We also looked for mutations associated with resistance to neuraminidase inhibitor treatment.

Methods

Patients and specimens

We included in the study 14 patients who were diagnosed with A/H7N9 infection at the Shanghai Municipal Centre for Disease Control and Prevention (CDC), Shanghai, China, and had had oseltamivir treatment for less than 2 days before admission to the Shanghai Public Health Clinical Centre (SPHCC) between April 4, and April 27,

2013. Table 1 outlines the demographic details, comorbidities, antiviral, and corticosteroid treatment received, and final disposition of these patients. Most patients received oseltamivir with doses of 75 mg or 150 mg twice daily for 5–20 days (median 13, IQR 5–11); four patients also received peramivir and four patients received corticosteroid therapy. These 14 patients do not include the two patients from Shanghai previously reported by Gao and colleagues.² All patients provided written informed consent to be enrolled into this study.

After admission, throat swabs were again obtained from every patient to confirm a diagnosis of A/H7N9 infection

	Age (years)	Sex	Comorbidities	Date of disease onset	Time from disease onset to admission (days)	Time from disease onset to initiation of antiviral therapy (days)	Antiviral regimen (time after disease onset)	Corticosteroid (methylprednisolone) (time after disease onset)	Outcome (time after disease onset)
ECMO (patients 1–3)									
1	74	Male	Cardiac disease; hepatitis B	Mar 31	6	5	Oseltamivir 75 mg twice daily (day 5–11)	None	Death (day 11)
2	88	Male	Hypertension; diabetes; COPD	April 10	7	6	Oseltamivir 150 mg twice daily (day 6–10); peramivir 0.6 g once daily (day 10–16)	40 mg once daily (day 7–8); 40 mg twice daily (day 9–16)	Death (day 19)
3	56	Male	None	April 2	2	2	Oseltamivir 75 mg twice daily (day 2–10); 150 mg once daily and 75 mg once daily (day 11–12); 150 mg twice daily (day 13–15); peramivir 0.6 g once daily (day 16–20)	40 mg every 8 h (day 7); 40 mg once daily (day 8–12)	ECMO dependent as of May 18 (day 46)
Mechanical ventilation (patients 4–7)									
4	81	Female	Cardiac disease	April 4	5	4	Oseltamivir 75 mg twice daily (day 4–8); 150 mg once daily and 75 mg once daily (day 9–19)	None	Discharge (day 22)
5	79	Female	Cardiac disease; hepatitis B	April 11	6	5	Oseltamivir 75 mg twice daily (day 5); peramivir 0.6 g once daily (day 6–16)	None	Under treatment as of May 18 (day 37)
6	62	Male	Hypertension	April 1	8	8	Oseltamivir 75 mg twice daily (day 8–14); 75 mg once daily (day 15–26)	None	Discharge (day 35)
7	78	Male	Hypertension	April 4	9	10	Oseltamivir 75 mg twice daily (day 10–14); 150 mg twice daily (day 15–22)	40 mg once daily (day 11–19)	Discharge (day 31)
Pneumonia (patients 8–14)									
8	74	Female	Cardiac disease; hypertension; diabetes	April 13	7	7	Oseltamivir 150 mg twice daily (day 7); peramivir 0.6 g once daily (day 8–13)	None	Discharge (day 22)
9	53	Male	None	April 7	6	5	Oseltamivir 75 mg twice daily (day 5–14)	None	Discharge (day 14)
10	75	Female	Cardiac disease; hypertension; diabetes	April 1	9	7	Oseltamivir 150 mg twice daily (day 7–11); 150 mg once daily and 75 mg once daily (day 12–26)	None	Discharge (day 32)
11	68	Male	Hypertension	April 4	7	5	Oseltamivir 75 mg twice daily (day 5–17)	None	Discharge (day 17)
12	74	Male	COPD	April 4	9	7	Oseltamivir 75 mg twice daily (day 7–17)	40 mg once daily (day 10–15)	Discharge (day 21)
13	47	Male	None	April 11	5	4	Oseltamivir 75 mg twice daily (day 4–11)	None	Discharge (day 16)
14	65	Male	Hypertension	April 1	5	3	Oseltamivir 75 mg twice daily (day 3–16)	None	Discharge (day 17)

ECMO=extracorporeal membrane oxygenation. COPD=chronic obstructive pulmonary disease.

Table 1: Demographic details, therapy, and outcome of patients with A/H7N9 infection

by real-time PCR (rtPCR) with primers targeting the H7 hemagglutinin and N9 neuraminidase with the reagents supplied by China CDC. Additional throat swabs, stool, urine, and blood samples were obtained from every patient daily after admission whenever possible. Throat swabs were placed in virus transport medium (minimum essential medium [MEM] with 2% fetal bovine serum [FBS] 5% penicillin/streptomycin and amphotericin B) immediately after collection and subsequently stored at -80°C . The serum, urine, and stool samples were also stored frozen at -80°C . The study protocol has been approved by the Ethics Committee of the SPHCC.

Procedures

The methods for the quantitative assay of viral RNA, viral genome sequencing by the Sanger method and by high-throughput 454 GS FLX sequencing (Roche, Mannheim, Germany), and genotype-specific Taqman assay (Takara, Dalian, China) to differentiate arginine (Arg) 292 lysine (Lys) mutation in the NA gene are described in the appendix. Since virus isolation might lead to selection of variant viruses that might not fully represent the virus population in the clinical specimen, we characterised the viral genome directly from the clinical specimens rather than from cultured isolates. We did a Sanger and high-throughput 454 GS FLX sequencing on the original clinical specimens. Our gene sequences are deposited in GenBank: accession number KF028373-KF028392 for Sanger sequence and access number PRJNA202283 for 454 sequence.

Statistical analysis

We analysed virus genome copy numbers (viral loads) after \log_{10} transformation. For statistical purposes, we used the lower detection limit of the assay (100 cDNA copies per mL) in cases of negative test results of virus load measurements. We used the Mann-Whitney U, Fisher's exact tests, and one-way ANOVA for group comparisons. For all analyses, we deemed a p value derived from a two-tailed test lower than 0.05 to be significant. We did all statistical analyses with SPSS 14.0 (SPSS Inc, Chicago, USA).

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The 14 patients in our study group had a median age of 74 years (IQR 60–78), ten of them (71%) were male (table 1). None of the patients were epidemiologically linked. Seven (50%) patients developed pneumonia and were oxygen-dependent but did not require ventilator support or ECMO; they constitute the pneumonia group. Four other patients further deteriorated with

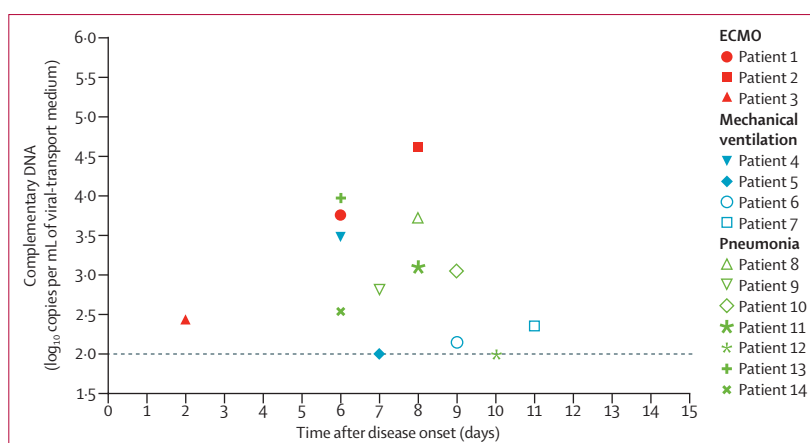


Figure 1: A/H7N9 viral load in the first available throat swabs from patients after admission
A/H7N9=influenza A subtype H7N9 virus. ECMO=extracorporeal membrane oxygenation. The dashed line denotes the limit of detection ($2 \log_{10}$ copies per mL).

acute respiratory distress syndrome (ARDS) and required mechanical ventilation (those constitute the mechanical ventilation group). The other three patients who developed pneumonia deteriorated rapidly even after treatment with neuraminidase inhibitor and mechanical ventilation and required extracorporeal membrane oxygenation (ECMO); two of them died (all three constitute the ECMO group).

The appendix shows the clinical and laboratory findings. In view of the small sample size, the absence of significance might not exclude differences between the groups. However, the three groups did not seem to differ significantly by age, days from onset to hospital admission or commencement of oseltamivir therapy (appendix). Laboratory findings at admission showed lymphocytopenia, and elevated lactic dehydrogenase, creatinine kinase, and C-reactive protein (appendix).

At admission and during subsequent treatment, we measured A/H7N9 viral load in throat swab and stool supernatants, serum, and urine, which were expressed as \log_{10} cDNA copies per mL. Since patients received neuraminidase inhibitor treatment on diagnosis, we could not study the natural history of the disease and viral load in the absence of antiviral therapy. However, since patients with A/H7N9 were admitted to SPHCC at varying times after disease onset, the viral load in throat swabs collected soon after admission from the patient group taken as a whole provides an aggregate profile of viral load over the course of illness before prolonged therapeutic intervention. Therefore, we plotted the viral load in the first available throat swab taken after admission for each patient in relation to duration of illness (figure 1). High viral load persisted in the throat through the first 10 days of illness in the absence of specific treatment (figure 1).

The median of viral loads in throat swabs taken at admission in the three groups of patients were 3.76 (range 2.43–4.61, IQR 2.43–4.61) in the ECMO group, 2.26 (2.00–3.49, 2.04–3.21) in the mechanically

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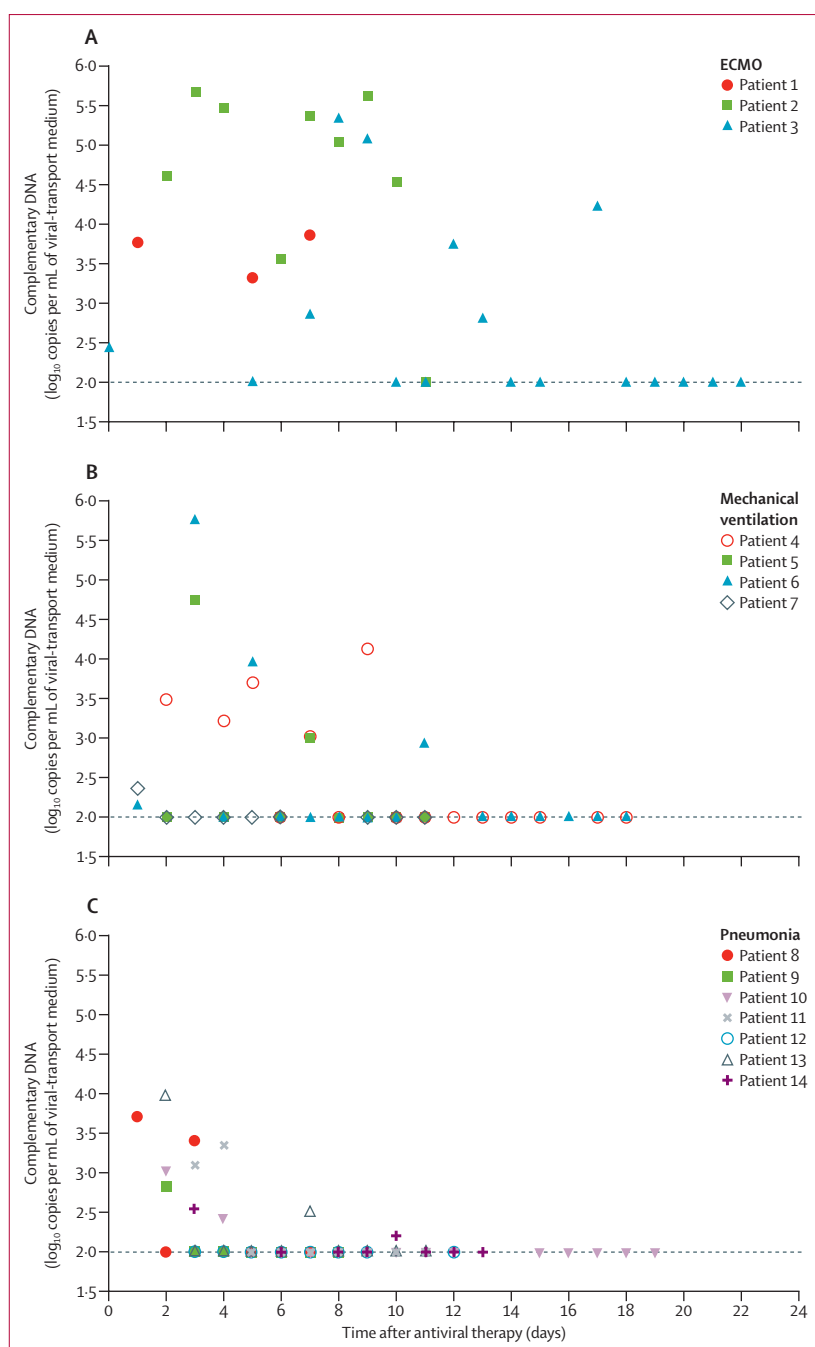


Figure 2: The kinetics of A/H7N9 viral loads in sequential throat swabs from patients on antiviral treatment A/H7N9=influenza A subtype H7N9 virus. ECMO=extracorporeal membrane oxygenation. The serial viral loads of individual patients from the (A) ECMO, (B) mechanical ventilation, and (C) pneumonia groups are plotted. The grey dashed line denotes the limit of detection (2 log₁₀ copies per mL).

ventilated group, and 3.05 (2.00–3.97, 2.54–3.71) in the pneumonia group (appendix) and did not differ between groups ($p=0.22$). When we compared the peak viral loads in swabs during the course of oseltamivir therapy, mean viral load was 5.34 (range 3.86–5.67, SD 0.96) in the ECMO group; 4.44 (2.36–5.76, 1.43) in the mechanically

ventilated group, and 3.05 (2.00–3.97, 0.68) in the pneumonia group (appendix). The mean peak viral loads of the ECMO group differed from those in the pneumonia group ($p=0.033$). A fitted linear regression model of viral load (response variable) against calendar time (predictor variable) assuming a shared slope within each of the three patient groups and a different intercept for every patient showed significant differences between the ECMO group and the pneumonia group ($p=0.047$), and between the ECMO group and the combination of the mechanical ventilation and pneumonia groups ($p=0.02$; appendix). In patients 2 and 3, ECMO was commenced on day 8 and day 19 after the start of oseltamivir therapy, and this intervention is unlikely to have significantly affected viral shedding of viral loads being compared here.

The peak viral load during hospital stay was higher in those who were more severely ill (ECMO and mechanical ventilation groups) than in those in the pneumonia group. We noted prolonged and high viral load after start of oseltamivir treatment in those with progressive illness leading to dependence on ECMO (figure 2).

Since we noted no apparent virological response to oseltamivir treatment in the patients of the ECMO group, we investigated whether emergence of resistance to oseltamivir might have accounted for the failure of therapy and ultimately, to clinical deterioration. We sequenced the NA and HA genes of clinical specimens from three patients in the ECMO group and two patients in the mechanically ventilated group using 454 Roche FLX. We obtained 1150468 reads, from which 226496 (19.7%) reads were mapped to the reference sequence A/Shanghai/2/2013. The strategy, using specific primer enrichment and random priming amplification, resulted in a higher depth in the two ends of each segment than in the internal segments (data not shown). The HA and NA sequences derived from these patients were highly homologous to the A/Shanghai/2/2013 strain, including Leu226 in the HA protein (H3 numbering position).

We noted the oseltamivir-resistance associated Arg292Lys mutation (position 292 with N2 numbering; position 294 with N9 numbering) in NA protein in the specimens from two of three patients in the ECMO group (patients 2 and 3) and in neither of the two patients tested in the mechanically ventilated patient group (table 2). Importantly, we detected a mixed population of arginine and lysine at position 292 from the NA gene in the sample from patient 2 obtained 7 days after oseltamivir therapy. To confirm these findings, we used Sanger sequencing to partially characterise the NA gene from original specimens, spanning the region containing aminoacid residue 292 from these patients at different timepoints of illness. Additionally, we obtained Sanger sequence data for one additional patient from the mechanical ventilation group (patient 4) and for two patients from the pneumonia group (patients 9 and 11; table 2). The results show that all the patients in the mechanically ventilated and pneu-

monia groups retained the wild-type Arg292. Three samples obtained from patient 2 in the ECMO group on days 4, 7, and 9 after starting oseltamivir treatment contained Lys292 mutation as the dominant population. In patient 3 (ECMO group), oseltamivir was started on day 2 of illness, and the specimen obtained 2 days later remained wild-type (Arg292), whereas we noted the Lys292 resistance mutation to be the dominant population in a sample obtained 9 days after treatment (table 2). These findings were supported by a genotype specific Taqman assay to differentiate NA Arg292Lys mutants in the NA gene, which showed a 32:68 mixed Arg/Lys population at day 3 after treatment, increasing to a predominant (96%) Lys292 population at day 7 after treatment. In patient 3, the specimen had 100% NA Arg292 at day 2 and 100% Lys292 at day 9 (appendix).

In patient 2 the increase in dominance of NA Lys292 mutation on day 7 after treatment seem to be associated with a rebound in viral load at this time (figure 2A). The emergence of Lys292 in patient 3 by day 9 (there were no specimens collected between day 2 and 9) coincided with a rebound in viral load (figure 2A). The detailed clinical case reports of patients 2 and 3 are shown in the appendix. Additionally, an Arg152Lys NA mutation was noted in a sample taken from patient 6 (from the mechanical ventilation group) on day 5, after oseltamivir treatment.

We detected viral RNA in the serum obtained at some time during the clinical illness of 12 (86%) of the 14 patients (appendix; all three patients in the ECMO group, all four patients in the mechanical ventilation group, and five of seven patients in the pneumonia group). We detected viral RNA in the urine of two of three patients in the ECMO group, all four patients in the mechanical ventilation group, and in five of the seven patients in the pneumonia group (appendix). Additionally, in six patients, viral RNA was still detectable in urine even after their throat swabs were negative for virus RNA. Furthermore, stool samples from most of our patients (12 of 14) had evidence of viral RNA, with nine patients still positive for virus RNA in stool samples 1–15 days (median 4 days) after the throat swabs became negative for virus RNA.

Discussion

We investigated the relation of clinical and virological factors associated with adverse clinical outcome in A/H7N9 infection in a cohort of 14 patients admitted to the Shanghai Public Health Clinical Centre, China. We noted that oseltamivir treatment was associated with falling viral load in the respiratory tract in most patients with A/H7N9 infection. We also report that the emergence of mutations associated with resistance to neuraminidase inhibitors in some patients with A/H7N9 infection is associated with treatment failure and adverse clinical outcome (panel).

The demographics of this patient group was broadly similar to that of the first 82 patients reported by Li and

	Clinical outcome (time after disease onset)	Days from disease onset to oseltamivir therapy	Viral load at admission (log 10 copies per mL)	Sampling time after oseltamivir treatment (days)	Aminoacid at NA residue 292	
					Sanger sequencing	454 sequencing
ECMO						
1	Death (11 days)	6	3.76	1; 6	Arg at day 1; Arg at day 6	Arg (13*) at day 1; unavailable at day 6
2	Death (19 days)	7†	4.61	4; 7; 9	Lys at day 4; Lys at day 7; Lys at day 9	Unavailable at day 4; Lys/Arg (5;1) at day 7; unavailable at day 9
3	Hospital admission‡	2	2.43	2; 9	Arg§ at day 2; Lys at day 9	Unavailable at day 2; Lys (13) at day 9
Mechanical ventilation						
4	Discharge (22 days)	5	3.49	2; 9	Arg at day 2; Arg at day 9	Unavailable at day 2; unavailable at day 9
5	Hospital admission‡	6	Und¶	3	Arg	Arg (2)
6	Discharge (35 days)	9	2.15	3; 5	Arg at day 3; Arg at day 5	Unavailable at day 3; Arg (32) at day 5
Pneumonia						
9	Discharge (14 days)	5	2.82	2	Arg	..
11	Discharge (17 days)	5	3.11	3	Arg	..

NA=neuraminidase gene. Arg=arginine. Lys=lysine. *Count of mapped reads. †Patient also received peramivir therapy (see table 1). ‡Patients 3 and 5 are still in hospital and the date of disease onset was April 2 for patient 3, and April 11 for patient 5. §Detected from sputum sample. ¶Und=below detection limit. ||Mutation R152K in NA was also detected in this sample.

Table 2: Emergence of the NA R292K mutation that confers resistance to NA inhibitors

Panel: Research in context

Systematic review

We searched PubMed on May 10, 2013, using the search terms “influenza” AND “H7N9” AND “oseltamivir resistance” or “viral load” and found no published articles. A separate search for “R292K” or “R294K” mutations identified 25 reports, but none of these were related to the novel H7N9 viruses. Searching through the text of reports published on the novel H7N9 virus, we found two papers^{2,3} that mention the Arg292Lys (equivalent Arg294Lys with N9 numbering) mutation in A/Shanghai/1/2013 virus.

Interpretation

Neither duration of illness before admission nor viral load in throat swabs at admission was associated with response to neuraminidase inhibitor treatment or clinical outcome. Patients who survived seemed to have decreasing viral load in throat swabs in response to treatment with neuraminidase inhibitors whereas those who deteriorated and became dependent on extracorporeal membrane oxygenation (ECMO) had persistently high viral loads. We showed that the emergence of the Arg292Lys mutation in the neuraminidase (NA) gene seems to be associated with failure of response to neuraminidase inhibitors and to adverse clinical outcome. The contribution of corticosteroid therapy in facilitating the emergence of Arg292Lys mutation or directly affecting a rebound of viral load, thereby leading to adverse clinical outcome, also needs consideration.

colleagues⁵ from China. Viral load in the throat swab of each patient at admission provides an insight into the overall viral load profile of human A/H7N9 infection over the course of the illness (figure 1). By contrast to pandemic influenza where viral load in the respiratory tract

rapidly declines within a few days of disease onset,⁶ A/H7N9 infected patients seem to have high viral load through the first week of the infection, which is reminiscent of patients with H5N1 disease.⁷ This high viral load is probably due to the absence of previous immunological memory for A/H7N9. It implies that, by contrast with seasonal influenza, antiviral treatment of A/H7N9 initiated many days after disease onset might still provide some clinical benefit.

Neither duration of illness before admission nor viral load in throat at admission correlated with response to oseltamivir therapy or adverse clinical outcome. We noted prolonged and high viral load after start of oseltamivir treatment in those with progressive illness leading to dependence on ECMO. Delay in viral clearance was also associated with the severity of the disease in patients with 2009 pandemic influenza A H1N1.⁸ The delayed viral clearance in patients with more severe disease might be due to the emergence of resistance to oseltamivir, as previously reported for H5N1 infection.⁹ We found the Arg292Lys mutation in two of three patients in the ECMO group; in one of these patients this mutation was only noted when the sample was taken 9 days after antiviral treatment started, a time that coincided with a rebound in viral load in that patient, suggesting that resistance emerged *de novo*, probably as a result of the oseltamivir therapy. This finding is similar to a previous report on two patients with H5N1, in which the emergence of oseltamivir resistance was associated with a failure to clear virus RNA from throat swabs and with a fatal outcome.⁹ Patients 2, 3, 7, and 12 also received corticosteroid treatment at days 1, 5, 1, and 3, respectively, after commencement of antiviral therapy. The start of corticosteroid therapy also preceded the increase in viral load in patients 2 and 3 and preceded the emergence of the Arg292Lys mutation in patient 3. The contribution of corticosteroid therapy to the emergence of the Arg292Lys resistance mutation, the increasing viral load and adverse clinical outcome also needs to be considered. Patients 7 and 12 who also received corticosteroid treatment did not have a rebound in viral load, emergence of Arg292Lys mutation, or an adverse clinical outcome.

Clinically, the Arg292Lys mutation in seasonal H3N2 influenza virus is known to confer resistance to oseltamivir.¹⁰ This same mutation in an H1N9 virus confers resistance to zanamivir and oseltamivir.¹¹ This mutation was also present in the cultured virus isolate A/Shanghai/1/2013 from a previously reported patient infected with the novel A/H7N9 virus.^{2,12} We have shown that A/Shanghai/1/2013 (H7N9) virus isolate contains a mixed population of Arg/Lys at position 292 of the NA gene, and by purifying virus plaques that carry NA Arg292 and Lys292, we have noted that this mutation increases resistance to oseltamivir by 100-fold and zanamivir by 30-fold in a fluorescence-based NA inhibition assay (unpublished data).

In patient 6 (from the mechanical ventilation group), we found emergence of Arg152Lys mutation in the NA gene. This mutation was first reported from an immunocompromised patient infected with influenza B after zanamivir treatment.¹³ Using the baculovirus expressed N9 NA protein, it had been shown to exhibit mild resistance to both zanamivir and oseltamivir *in vitro*.¹⁴

Several serial specimens of serum, faeces, and urine from the 14 patients allowed us to detect virus RNA from these specimens in many patients, but we found no correlation with clinical outcome. Virus RNA in serum has also been detected in patients with severe pandemic H1N1 2009 infection (17.3%)⁸ and in H5N1-infected patients (56%).⁷ Influenza virus was previously detected in the stool of patients with seasonal, pandemic H1N1 and avian H5N1 influenza.^{9,15} The A/H7N9 virus detected in stool might result from swallowed respiratory secretions. Viral RNA might represent non-infectious virus rather than infectious virus particles, but it is important to investigate whether A/H7N9 virus disseminates beyond the respiratory tract.

In conclusion, oseltamivir treatment, even when started 48 h or more after disease onset, was associated with falling viral load in the respiratory tract in most patients with A/H7N9 infection. Therefore, early treatment of suspected or confirmed cases is strongly encouraged. The emergence of an NA Arg292Lys mutation in two patients infected with A/H7N9 was temporally associated with a rebound of virus load, treatment failure, and a poor clinical outcome. The role of corticosteroids in facilitating the emergence of the NA Arg292Lys mutation or even directly affecting viral load and adverse clinical outcome deserves consideration. The fitness and stability of the Arg292Lys mutation in A/H7N9 needs to be investigated. The apparent ease with which antiviral resistance emerges in A/H7N9 viruses is concerning; it needs to be closely monitored and considered in future pandemic response plans.

Contributors

ZY and MP were coprincipal investigators, designed and supervised the study, and applied the grants. YH, SL, ZS, WW, PH, JL, and XnZ contributed equally to this work. SL, XX, LZa, TL, and ZZg had roles in clinical management and data collection. XnZ and SW obtained clinical laboratory testing data for analysis. YH, ZS, LX, YLu, DT, XgZ, ZZu, JH, and BS did viral detection and viral load quantitation. WW, PH, WG, H-LY, KH, HC, XL, JP, LZg, BK, and YLi did the sequencing and analysis. YH, JL, XnZ, and WW drafted the manuscript, and all authors contributed to review and revision and have seen and approved the final version.

Conflicts of interest

We declare that we have no conflicts of interest.

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