

Outcomes of adults hospitalised with severe influenza

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ABSTRACT

Background The aim of this study was to investigate factors affecting clinical outcomes of adults hospitalised with severe seasonal influenza.

Methods A prospective, observational cohort study was conducted over 24 months (2007–2008) in two acute, general hospitals. Consecutive, hospitalised adult patients were recruited and followed once their laboratory diagnosis of influenza A/B was established (based on viral antigen detection and virus isolation from nasopharyngeal aspirates collected per protocol). Outcomes studied included in-hospital death, length of stay and duration of oxygen therapy. Factors affecting outcomes were analysed using multivariate Cox proportional hazards models. Sequencing analysis on the neuraminidase gene was performed for available H1N1 isolates.

Results 754 patients were studied (influenza A, n=539; >75% H3N2). Their mean age was 70±18 years; co-morbidities and serious complications were common (61–77%). Supplemental oxygen and ventilatory support was required in 401 (53.2%) and 41 (5.4%) patients, respectively. 39 (5.2%) patients died; pneumonia, respiratory failure and sepsis were the causes. 395 (52%) patients received antiviral (oseltamivir) treatment. Omission of antiviral treatment was associated with delayed presentation or negative antigen detection results. The mortality rate was 4.56 and 7.42 per 1000 patient-days in the treated and untreated patients, respectively; among those with co-morbidities, it was 5.62 and 11.64 per 1000 patient-days, respectively. In multivariate analysis, antiviral use was associated with reduced risk of death (adjusted HR (aHR) 0.27 (95% CI 0.13 to 0.55); p<0.001). Improved survival was observed with treatment started within 4 days from onset. Earlier hospital discharge (aHR 1.28 (95% CI 1.04 to 1.57); p=0.019) and faster discontinuation of oxygen therapy (aHR 1.30 (95% CI 1.01 to 1.69); p=0.043) was associated with early treatment within 2 days. Few (n=15) H1N1 isolates in this cohort had the H275Y mutation.

Conclusions Antiviral treatment for severe influenza is associated with reduced mortality and improved clinical outcomes.

INTRODUCTION

It has been estimated that 5–10% of the world's population are affected by seasonal influenza annually, causing 3–5 million severe infections and 250 000–500 000 deaths.¹ In North America, seasonal influenza causes excess hospitalisations in 230–1670 per 100 000 persons aged >65 years, 32 000 respiratory/cardiovascular deaths and 43 000

all-cause deaths annually.^{2–5} Persons with chronic medical conditions (eg, pulmonary, cardiovascular, liver, renal and neurological diseases, diabetes or immunosuppression) have a >30-fold increase in risk of hospitalisation and death.⁶ In Hong Kong, a similar situation exists.^{7–9} Among the circulating seasonal influenza subtypes, H3N2 is usually a more frequent cause of severe illness and hospitalisation.^{2–4} In 2009, a novel influenza A/H1N1 virus emerged and rapidly caused a pandemic.^{10–11} By November, 622 482 laboratory-confirmed pandemic influenza A/H1N1 infections and 8768 deaths were reported to the WHO.¹⁰ The pressure on medical facilities and intensive care units due to severe influenza infections has been substantial.^{12–14}

Despite the high disease burden, there are many uncertainties concerning the clinical outcomes of and the most appropriate management approach to patients hospitalised with influenza. Influenza is frequently underdiagnosed in the acute care hospital setting, as its presentations are diverse and the clinical scenarios are often complex in adults.^{15–17} Patients may present late with serious complications, which result in the requirements for oxygen/ventilatory support, intensive care, prolonged hospitalisations or even deaths.^{15–18–25} The lack of data may have reflected the difficulties in performing randomised placebo-controlled trials in these clinical settings. We therefore aimed to describe the diagnosis, treatment and outcomes of a large cohort of hospitalised patients with influenza. Factors associated with outcomes were then analysed.

METHODS

Study population

A prospective, observational cohort study was conducted among hospitalised adults with laboratory-confirmed, seasonal influenza infection. Patients aged ≥18 years admitted to two hospital medical departments between 1 January 2007 and 31 December 2008 (24 months) were recruited. The two participating hospitals are acute, general public hospitals operating under the Hospital Authority of Hong Kong, which serve an urban population of >1.5 million. The Hospital Authority is the main provider of acute medical services in Hong Kong.¹⁹

During the study period, influenza infection in hospitalised adults was diagnosed according to a standard protocol (see next section). Consecutive patients were identified and recruited by the laboratory and clinical research teams, on a daily basis, once the diagnosis of influenza A or B was established.^{19–25–26} Clinical data were recorded using a standard tool, and continued until discharge or

death during the same hospital stay. Six patients involved in concurrent intervention studies were excluded from the analyses. Ethical approval for the study was obtained from the Institutional Review Boards of the Hospital Authority of Hong Kong and The Chinese University of Hong Kong.

Case finding and management of influenza infection

Procedures of hospitalisation of adult patients with influenza have been described.^{19 25 26} In brief, patients presenting with symptoms of acute febrile respiratory illnesses were admitted if they developed potentially serious medical conditions and/or if their exacerbation of their chronic illnesses or their severe symptoms were considered to be impossible to be managed at home. Nasopharyngeal aspirate (NPA) was collected at presentation from all such patients, regardless of perceived aetiology, to test for influenza A and B infections using an immunofluorescence assay (IFA) or an immunochromatographic assay (ICA), followed by virus isolation (see next section).^{19 25–27} The results of IFA/ICA were generally available to clinicians within a few hours to assist management. Bacterial culture of sputum samples was also routinely performed.

Based on local recommendations,²⁸ antiviral treatment would be considered for patients hospitalised with confirmed influenza and presenting within 48 h from onset. In this study, there were no restrictions on the choice of antivirals. Oseltamivir, if given, was prescribed according to the standard dosing regime (75 mg twice daily orally, for 5 days) upon diagnosis, as previously described.^{19 25} Dosage adjustment, if necessary, was made according to the patient's renal function (75 mg daily, if creatinine clearance <30 ml/min). For patients who presented later than 2 days, the managing physicians were allowed to make decisions regarding antiviral use. Since this study involved no intervention, patients were managed and discharged according to usual clinical practice.¹⁹

Data collection and definitions

Patients were considered to have laboratory-confirmed influenza infection if they had positive results from the influenza antigen-specific IFAs/ICAs, and/or with influenza virus isolated from the same NPA samples.^{19 25 26}

Once recruited, clinical information was collected systematically by designated research staff using a standardised data collection form, which included demographics, medical comorbidities, symptom/fever onset time, complications developed, requirement for oxygen therapy and ventilatory support, details of antiviral treatments, clinical responses and outcomes.^{19 25 26} Oseltamivir treatment was defined as at least 1 day of treatment received by a patient. The time interval between symptom onset and the administration of the first dose of oseltamivir treatment was calculated for each patient.

The main outcome measure was all-cause death that occurred during the same hospital stay. Secondary outcome measures included (1) total duration of hospitalisation related to the current episode of influenza; (2) duration of supplemental oxygen therapy, if required (or in the case of pre-existing long-term oxygen therapy, return to the patient's usual oxygen requirement); and (3) duration of fever during hospitalisation. Resolution of fever was defined as a recorded tympanic temperature <37.5°C for at least 1 day.

Virological investigations

All NPAs collected were subjected to viral antigen detection for influenza A and B, based on IFA or ICA. High specificity has been reported with such assays for influenza; false positives are

rare.^{19 23–27} Concomitantly, virus isolation was performed for all NPA samples using Madin–Darby canine kidney (MDCK) cells, as previously described.^{19 23 27} All influenza isolates were submitted to the National Influenza Centre at the Centre for Health Protection in Hong Kong for further characterisation including subtyping into H1 and H3. All virological investigations were performed under Biosafety Level II containment.^{19 25 26}

Sequencing analysis on the neuraminidase gene was conducted for all available H1N1 isolates collected since January 2008 (n=49) to detect the oseltamivir-resistant-associated mutation (H275Y). Prior isolates were not examined as the H275Y mutant was not found in Hong Kong before 2008.^{29–31} Details of the laboratory methods are provided in appendix 1.

Statistical analysis

The Student t, Mann–Whitney U, Fisher exact and χ^2 tests with continuity correction were used for univariate comparisons whenever appropriate. Variables with p values <0.2 in the univariate analyses were entered into multivariate Cox proportional hazards models (backward, stepwise) to determine independent factors associated with death that occurred within 21 days of hospitalisation.¹⁹ The variables 'time to presentation' and 'hospital site' were also included in the analyses. Adjusted HRs (aHRs) and 95% CIs were calculated for each explanatory variable. An aHR <1 indicated a lower chance of death. Survival function (curve) was also developed to show the effect of explanatory variables on time to death based on the final model. Multivariate Cox proportional hazards models were also used to analyse independent factors associated with hospital discharge or discontinuation of supplemental oxygen therapy. An adjusted HR <1 indicated a lower chance of attaining these outcomes. A multiple linear regression model (backward) was used to identify independent factors affecting duration of fever during hospitalisation; the regression coefficient (β), SE and the 95% CI were reported for explanatory variables.²⁵

In all analyses, a p value of <0.05 was considered to indicate statistical significance. All probabilities were two tailed. Statistical analysis was performed using PASW Statistics software, version 17.0.³²

RESULTS

Clinical description of cohort

A total of 754 patients were studied (influenza A 71%, B 29%; >75% of influenza A being subtype H3N2), with 442 and 312 patients being recruited in 2007 and 2008, respectively. The median monthly positive influenza virus detection rate during each peak season was 11.5% (IQR 7.0%–16.0%). Their demographics, underlying conditions, laboratory diagnoses, medical complications, antiviral use and clinical outcomes are summarised in tables 1 and 2. Patients were mostly of older age (mean±SD, 70±18 years), and the majority had underlying chronic illnesses (60%). One or more medical complications related to influenza infection were identified in 77% at presentation. Pneumonia (325 episodes), acute exacerbation of chronic obstructive pulmonary disease (COPD) or asthma (140 episodes), acute bronchitis (41 episodes) and cardiovascular complications such as congestive heart failure, acute coronary syndrome and atrial fibrillation (77 episodes) were among the most frequently diagnosed conditions. In 7% of cases, fever was never documented during the course of hospitalisation; in 16%, patients had negative antigen detection results, but later influenza was confirmed with positive viral cultures.

Table 1 Patient characteristics and laboratory diagnosis of adults hospitalised with influenza, 2007–2008 (n=754)

Patient characteristics	n (%)
Age ≥70 years	454 (60.2)
Gender, male	372 (49.3)
Nursing home residents	141 (18.7)
Co-morbidity, any*	456 (60.5)
Major co-morbidity (congestive heart failure, cerebrovascular, neoplastic, chronic liver and renal diseases, ischaemic heart diseases, diabetes mellitus and use of immunosuppressants)	364 (48.3)
Chronic lung diseases (COPD, asthma, bronchiectasis and pulmonary fibrosis)	166 (22.0)
Diagnoses	
Influenza A	539 (71.5)
Influenza B	215 (28.5)
Rapid diagnosis positive by IFA	558 (74.0)
Rapid diagnosis positive by ICA	74 (9.8)
Rapid diagnosis negative by IFA or ICA	122 (16.2)
Positive virus culture	667 (88.5)
Influenza A, subtype H3	414 (76.8)
Influenza A, subtype H1	56 (10.4)
Subtyping results unavailable	69 (12.8)

*Patients may have ≥1 co-morbid illness.^{19 25 26} Data on influenza vaccination status were only available in 322 patients; among these, 21.4% had received influenza vaccines prior to the 'flu' seasons (similar to our previous report).¹⁹ COPD, chronic obstructive pulmonary disease; ICA, immunochromatographic assay; IFA immunofluorescence assay.

A total of 39 (5.2%) patients died during the same period of hospitalisation (related to influenza A/H3N2=21; A/H1N1=2; A/ unspecified=3; B=13). Median time interval from admission to death was 7 (IQR 5–13) days. The main conditions contributing to death as diagnosed by the managing physicians were: pneumonia (33 episodes), respiratory failure related to underlying COPD/asthma (11 episodes), sepsis (8 episodes) and acute cardiovascular events (7 episodes). Among survivors, the median hospital length of stay was 5 (IQR 3–10) days. The median duration of oxygen therapy, if required, was 3 (IQR 2–6) days.

Management of patients

Supplemental oxygen therapy was given to 53% of patients, whereas 5% had required ventilatory support (intubation or non-invasive ventilation) for respiratory failure. Antibacterial treatment was used in 92% of all cases, and in 100% of fatal cases. Fifty-two per cent (n=395) of patients received oseltamivir treatment; no other antiviral use, including amantadine/rimantadine or zanamivir, was found in this cohort. Omission of antiviral treatment (n=359) was associated with delayed presentation >2 days from illness onset, or a negative viral antigen detection result (both $p<0.05$). The time interval from illness onset to diagnosis of influenza infection was 1.5 ± 1.2 days in the treated and 2.2 ± 1.6 days in the untreated patients, respectively ($p<0.001$). Oseltamivir treatment was initiated within 2 days from onset in 78%, and within 4 days in 95%. A total of 88 patients received treatment later than 2 days. Early discontinuation of oseltamivir was noted in 7 (1.8%) patients for the reasons listed in table 2 footnotes.

Analysis of outcomes

Univariate analyses indicated that older age, male sex, presence of co-morbid illnesses, being a nursing home resident and need for ventilatory support were factors associated with death. There was no significant difference in mortality observed between the two participating hospitals, or between influenza subtypes A and B. The unadjusted mortality rate among

Table 2 Complications, clinical outcomes and treatment prescribed for adults hospitalised with influenza, 2007–2008 (n=754)

Complications and clinical outcomes	n (%)
Complications, any*	581 (77.1)
Cardiorespiratory complication (pneumonia, acute bronchitis, acute exacerbation of underlying chronic respiratory conditions, heart failure, acute coronary syndrome, arrhythmia and acute cerebrovascular events)	493 (65.4)
Secondary bacterial infection†	68 (9.0)
Supplemental oxygen required	401 (53.2)
Ventilatory support required‡	41 (5.4)
Direct discharge from acute hospitals	562 (74.5)
Extended care in subacute hospitals	165 (21.9)
Duration of hospitalisation >5 days	319 (44.6)
Duration of hospitalisation >10 days	177 (24.8)
Death during the same hospital stay§	39 (5.2)
Treatment prescribed	
Antiviral use (oseltamivir)	395 (52.4)
Initiated ≤2 days from onset	307 (40.7)
Initiated ≤4 days from onset	377 (50.0)
Early discontinuation of antiviral¶	7 (1.8)
Antibacterial	693 (91.9)

*Patients may have ≥1 complication (see text).^{19 25 26} Other complications include syncope/presyncope, dehydration, renal impairment, decreased conscious level, confusion and metabolic disturbances (158 episodes).

†Sputum culture confirmed bacterial infection (*Streptococcus pneumoniae* 8, *Staphylococcus aureus* 8, *Haemophilus influenzae* 15, *Moraxella catarrhalis* 2, *Pseudomonas aeruginosa* 18, *Klebsiella* spp. 10, other Gram-negative bacilli 7, other Gram-positive cocci 3; mixed infection in a few cases) at presentation. Another 28 (3.7%) patients developed nosocomial infection (*P. aeruginosa*, *Acinetobacter* spp., other resistant enterobacteriaceae, methicillin-resistant *S. aureus*). The bacterial pathogen was identified in 31% of fatal cases (community-acquired 5, nosocomial 8). Antibacterials were given according to standard recommendations.^{19,48}

‡Intubated and received mechanical ventilation, or non-invasive ventilatory support.

§Death due to all causes during the same period of hospitalisation. 35 patients died within the first 3 weeks of hospitalisation.

¶Reasons for discontinuation of oseltamivir treatment (other than death): vomiting 1, dizziness 1, cannot tolerate oral medication 2, confusion in patients with underlying dementia/schizophrenia 3.

patients who had received oseltamivir treatment was 4.56 per 1000 patient-days, whereas that among patients who had never received antiviral treatment was 7.42 per 1000 patient-days. Among patients with major medical co-morbidities, the mortality rates were 5.62 and 11.64 per 1000 patient-days, respectively. Among patients who had required supplemental oxygen therapy, the mortality rates were 6.54 and 11.00 per 1000 patient-days, respectively.

In a final multivariable model (n=754), use of antiviral treatment was significantly associated with a reduced risk of death, with an aHR of 0.27 (95% CI 0.13 to 0.55; $p<0.001$) (table 3, figure 1A). Further analyses showed that the aHR for death associated with antiviral treatment initiated within 2 days from onset, versus no and delayed treatment, was 0.29 (95% CI 0.14, to 0.61; $p=0.001$); similarly, the aHR associated with treatment initiated within 4 days was 0.34 (95% CI 0.17 to 0.70; $p=0.003$).

The impact of timing of treatment initiation on mortality was evaluated (figure 1B). When compared with patients who received antiviral treatment within 2 days from onset (n=307) (aHR 1.00), those who did not receive treatment because of late presentation or negative antigen detection test results (n=194) had significantly higher risks of death (aHR 8.49 and 3.24, respectively; both $p<0.05$), whereas the risk of death in patients who received antiviral treatment despite presenting later than 2 days (n=88) was not shown to be statistically different in the final multivariate model (aHR 1.87; $p=0.296$). Subgroup analysis showed that when compared with untreated patients, those who received antivirals later than 2 days had better outcomes (appendix 2).

Table 3 Factors associated with death in patients hospitalised with confirmed influenza infection as shown in a final Cox proportional hazards model (n=754)

Variables	Adjusted HR (95% CI)	p Value
Oseltamivir treatment	0.27 (0.13 to 0.55)	<0.001
Age >70 years	2.94 (0.85 to 10.18)	0.088
Male sex	3.92 (1.80 to 8.57)	0.001
Major co-morbidity	2.28 (1.02, 5.09)	0.045
Ventilatory support	8.29 (3.97 to 17.29)	<0.001
Nursing home resident	7.62 (3.51 to 16.54)	<0.001

Refer to tables 1 and 2 for explanation of variables compared. The outcome analysed was death that occurred within 21 days of hospitalisation. Potential confounders including 'time from symptom onset to presentation', 'chronic lung diseases' and 'negative antigen test result' were adjusted for in this model. Being managed in either hospital site was not found to affect outcomes in the model. There was also no significant difference in mortality found between influenza A and B subtypes.

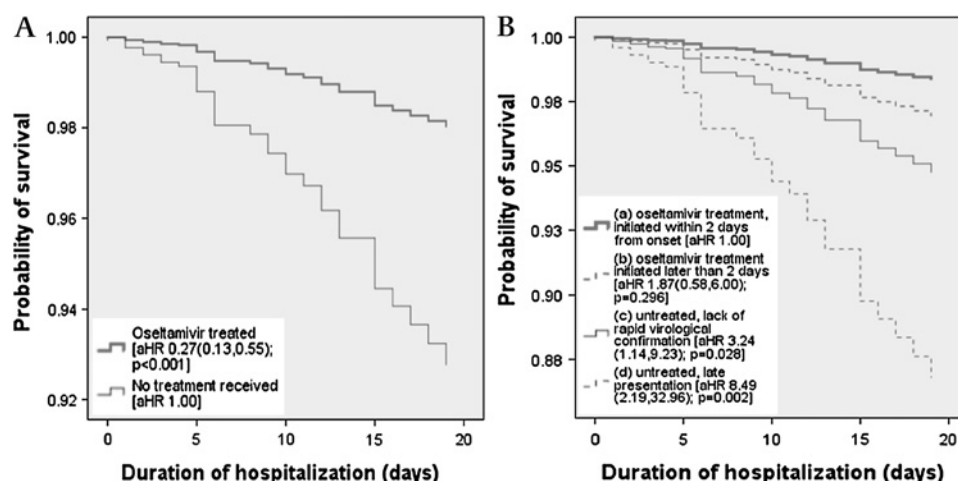
Factors affecting duration of hospitalisation were also evaluated. Patients who received antiviral treatment within 2 days from onset (n=307) were compared with those who received no treatment (related to late presentation or negative antigen detection results, n=194) or late treatment (n=88). Early initiation of antiviral treatment was found to be independently associated with earlier hospital discharge among survivors (aHR 1.28, 95% CI 1.04 to 1.57; $p=0.019$). A faster rate of weaning off supplemental oxygen therapy (aHR 1.30, 95% CI 1.01 to 1.69; $p=0.043$) may have contributed to their earlier discharge (figure 2A,B; appendix 3). Duration of fever was affected by the presence of pneumonia ($\beta +0.45$, SE 0.18, 95% CI 0.10 to 0.80; $p=0.012$), bacterial infection ($\beta +0.42$, SE 0.20, 95% CI 0.02 to 0.82; $p=0.040$) and corticosteroid administration for COPD/asthmatic exacerbation ($\beta -0.73$, SE 0.22, 95% CI -1.17 to -0.29 ; $p=0.001$); a trend can be shown for early oseltamivir treatment associated with resolution of fever ($\beta -0.27$, SE 0.17, 95% CI -0.60 to 0.07; $p=0.116$).

In this cohort, genotypic resistance testing showed that only 15 of the H1N1 isolates harboured the H275Y mutation (3% of all subtyped influenza A virus). All but one case were diagnosed in the latter half of 2008. Eight cases were not treated with oseltamivir. No patient had received ventilatory support, and there were no deaths.

DISCUSSION

Adults hospitalised with influenza have high morbidity and mortality. Our results provided evidence to suggest that use of antiviral treatment in such patients may improve survival.

Figure 1 Survival functions of patients hospitalised with influenza as shown in the final Cox proportional hazards models, after adjustment of confounders. Adjusted HR (aHR) >1 signifies a higher risk of death. (A) Use of antiviral treatment was associated with a higher probability of survival. Treatment initiated at all time intervals from onset was compared with no treatment. (B) Impact of timing of treatment initiation. Early antiviral treatment within 2 days from onset (a), late treatment (b) and omission of treatment due to lack of rapid virological confirmation (c) or late presentation (d) were compared. Early treatment was associated with the best survival; late treatment also seemed beneficial (see text and appendix 2).



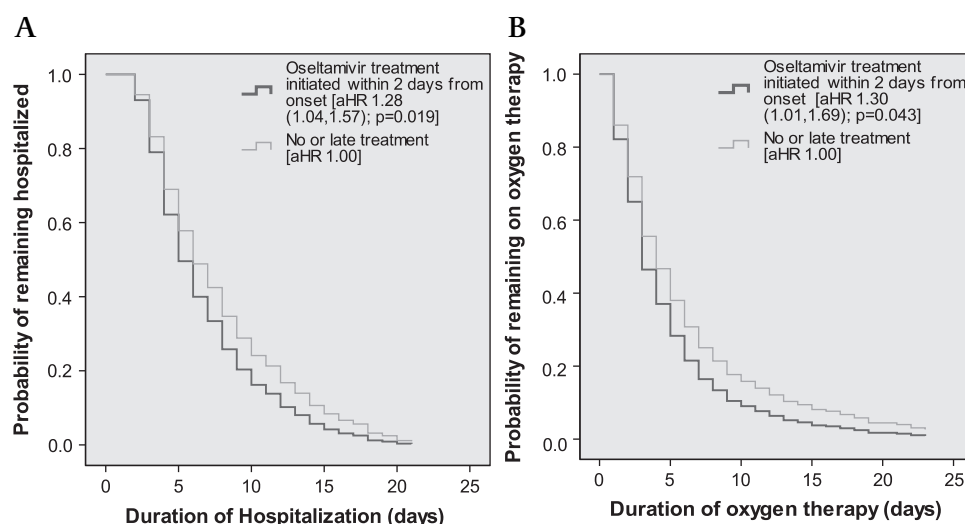
Greater benefits were shown with early treatment initiated within 2 days, which included mortality reduction, shorter duration of oxygen therapy and earlier discharge; but late treatment within 4 days also seemed beneficial.

It is known that use of neuraminidase inhibitors can shorten the duration of influenza illness and reduce lower respiratory complications in younger, non-hospitalised adults.^{2 23 33–35} This cohort study shows that such treatment may reduce mortality in older persons with co-morbidities who are hospitalised with severe and complicated infections.^{36–38} Our findings agree with two recent surveillance studies describing the high morbidity and mortality of seasonal influenza, and lower risks of death associated with antiviral treatment (OR 0.1–0.2)^{18 39} Although data are limited, survival benefit has also been reported for H5N1^{40 41} and pandemic influenza A/H1N1 infection with treatment.¹² Reduced hospital length of stay with early treatment is demonstrated, similar to our previous report¹⁹; however, earlier discontinuation of oxygen therapy in patients with cardiorespiratory complications, and possibly faster resolution of fever in such patients, are new findings.

Time of antiviral initiation may have an important impact on clinical outcomes. A larger reduction in mortality and faster rate of recovery could be seen in patients who received early (≤ 2 days) antiviral treatment^{12 39 40}; thus antivirals should be started as soon as the diagnosis is made. Although the magnitude of benefit seemed smaller, patients who had received treatment later than 2 days (but mostly within 4 days) were still observed to have lower mortality than those without treatment (figure 1b; and appendix 2).¹⁸ Treatment bias is unlikely to explain this finding, as there was a tendency to treat the more severely ill patients despite their late presentation. We have previously reported that influenza virus load in hospitalised patients may remain high beyond 48 h from onset; and that antivirals initiated within 96 h are effective in lowering viral loads.²⁵ Our findings therefore support the recent suggestion that antiviral treatment may still be considered in the severely ill, hospitalised influenza patients who present beyond 48 h from illness onset.^{10 23 42}

Since clinical diagnosis of influenza is inaccurate,^{16 17} an active case-finding approach was adopted.^{15 19 43} Initial confirmation of influenza infection was based on viral antigen detection (IFA, ICA), which had provided rapid results to assist clinical decisions in many cases. Although these tests are highly specific, they are only moderately sensitive; in our cohort, >15% of the cases tested negative, but later diagnosed by culture. An antiviral was

Figure 2 Effect of early antiviral treatment on (A) probability of remaining hospitalised by day 21 among survivors, and (B) probability of remaining on supplemental oxygen therapy, as shown in the respective final Cox proportional hazards models. Adjusted HR (aHR) >1 signifies a higher chance of discharge or discontinuation of supplemental oxygen. From the models, the adjusted median duration of hospitalisation in the early treatment group and the 'no or late treatment' group was 5 and 6 days, respectively; the adjusted median duration of oxygen therapy was 3 and 4 days respectively.



consequently not given; and omission of treatment in these patients was associated with deterioration. Whether these patients had lower viral loads (leading to false-negative antigen detection results)^{15 23 44–46} and how this might affect outcomes require further study. Nevertheless, our data suggest that a high index of suspicion and the application of more sensitive diagnostic assays such as reverse transcription–PCR (RT–PCR) are necessary to facilitate early therapeutic intervention.^{23 24 42 46} In 2009, we started to use RT–PCR to diagnose pandemic influenza A/H1N1 infection according to the latest recommendations.^{10 42} We also agree that in managing severely ill patients, clinicians should not delay treatment and wait for virological confirmation, especially when the prevalence of influenza is high (eg, seasonal peaks, pandemics), or when the more sensitive assays are not readily available.^{23 24 42}

The strengths of our study include a large sample size, recruitment of consecutive patients by two centres and that confounders were carefully adjusted in the analyses. There are several limitations. The study was non-randomised. Although we did not study reasons for omitting treatment other than lack of rapid diagnosis and late presentation, we believe that there was generally a higher intention to treat patients who were more seriously ill.²⁵ In this cohort, a significantly higher proportion of patients who required oxygen therapy had received oseltamivir (data not shown). While such bias can mask the beneficial effects, we are still able to observe lower mortality in those who were treated. Lack of treatment for secondary bacterial infection is unlikely to be the explanation, as all fatal cases (and 92% of all subjects) had received antibacterial treatment (table 2).^{12 14 18–21 47 48} Superimposed bacterial infection should always be suspected and treated; however, concomitant use of neuraminidase inhibitors to treat influenza also seems important to achieve better outcomes.^{49 50} The impact of oseltamivir resistance was probably very small, as most (83%) of our cases were either influenza A/H3N2 or the B subtype, which are considered susceptible^{10 23 51}; and among the H1N1 isolates only 15 were detected to have the resistant-associated H275Y mutation (all patients survived, and many did not receive oseltamivir).^{30 31 52} In Hong Kong, the mutant became solely predominant only in early 2009.^{29 30} Although we are not studying the pandemic A/H1N1 infection, our findings may contribute to the understanding of important management principles of severe influenza. Future directions of clinical research in hospitalised patients should include areas concerning an extended time window for therapeutic intervention, extended duration or high dose treatment, combination antiviral treatment

and parenteral antivirals, and the application of more sensitive diagnostics to assist clinical management.^{23–25 33 38 39 53}

In conclusion, our data suggest that antiviral treatment for severe influenza may reduce mortality and improve clinical outcomes in adult hospitalised patients. Prompt case recognition and accurate diagnosis should be ensured, and timely therapeutic intervention should be considered. The role of delayed treatment in the seriously ill may deserve further evaluation.

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Competing interests None.

Ethics approval This study was conducted with the approval of the Institutional Review Boards of the Hospital Authority of Hong Kong and The Chinese University of Hong Kong.

Contributors Conception and design: NL, KWC, PKSC. Analysis and interpretation of the data: NL, KWC, PKSC, DSCH, JJYS. Drafting of the article: NL. Critical revision of the article for important intellectual content: KWC, PKSC, DSCH, GCYL, JJYS. Final approval of the article: NL, KWC, PKSC, DSCH, RWML, JJYS. Patient enrolment, collection and assembly of data: NL, KWC, GCYL, BCKW, RYKW, WYS, WMH, RWML. Administrative, technical or logistic support: RYKW, PKSC, KLKN, RWML.

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APPENDIX 1

To detect H275Y mutation, RT–PCR (SuperScript One-Step RT-PCR System with Platinum Taq DNA Polymerase, Invitrogen, Carlsbad, California, USA) was carried out using 5 µl of RNA extracts and 0.2 µM of forward and reverse primers (N1-F299-323: 5'GGGCTATATACACAAAGACAACAG3' and N1-R1334-1310: 5'CTCCCACTAGTCAGATTGTGTAT3') in a 25 µl reaction mixture. The cycling conditions were an initial hold at 52°C for 30 min, and then 94°C for 2 min; followed by 40 cycles at 94°C for 30 s, 52°C for 30 s and 68°C for 70 s, and a final extension of 68°C for 10 min. The purified amplicons were sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, California, USA) using primers N1-F758-778 (5'CCGCTCGTACAAATCTTCA3') or N1 R978-958: (5'ACCGAACACTCCACTGCAGAT3') for the forward and reverse strands, respectively.

APPENDIX 2

Impact of timing of treatment initiation on mortality

- a. Whole cohort (n=754, figure 1B). When compared with antiviral treatment initiated within 2 days from onset (n=307) (aHR 1.00), omission of treatment because of lack of rapid virological confirmation or late presentation (n=194) was associated with significantly higher risk of death (aHR 3.24 and 8.49, respectively; both p<0.05). Patients who presented within 2 days but were not given antiviral treatment were also shown to have higher risks of death in the final model (n=165) (aHR 4.53 (95% CI 1.85 to 11.05); p=0.001) (curve not shown). Treatment initiated later than 2 days (n=88), however, was not shown to be associated with a significantly higher risk when compared with early treatment (aHR 1.87, p>0.05) in the model. Other variables that were found to be significant in the final Cox proportional hazards model included age >70 years, male gender, presence of co-morbidities, being a nursing home resident and need for ventilatory support.
- b. In a subgroup analysis, patients who received antiviral treatment later than 2 days from onset (n=88) were compared with all untreated patients (n=359). A Cox proportional hazards model showed that aHR for death in treated patients was 0.28 (95% CI 0.09 to 0.94; p=0.039), adjusted for demographic variables, co-morbidities and need for ventilatory support as described above.

APPENDIX 3

Analyses on length of stay and duration of oxygen therapy

- Patients who survived and remained hospitalised after 24 h of admission were included in these analyses. aHR >1 signifies a higher chance of discharge or weaning off oxygen therapy, whereas a ratio <1 signifies a lower chance of these outcomes.
- a. In this analysis, early oseltamivir treatment within 2 days from onset was independently associated with earlier hospital discharge (see text and figure 2A). Presence of co-morbidity, complications, need for ventilatory support, older age and need for extended care were found to be significantly associated with longer duration of hospitalisation in the final Cox model. Being managed in either study hospital was not found to affect length of stay.
 - b. In this analysis, early oseltamivir treatment within 2 days from onset was independently associated with a shorter duration of oxygen therapy (see text and figure 2B). Underlying chronic lung diseases, need for ventilatory support and older age were found to be significantly associated with longer duration of oxygen therapy in the final Cox model.