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Influenza virus load in hospitalised patients

Key Messages

1. Hospitalised patients with severe influenza have persistently high viral loads, for whom a different therapeutic approach may be needed.
2. Active screening of influenza infection should be performed in all high-risk patients hospitalised with febrile respiratory illness. Early diagnosis and treatment to suppress the high viral load may maximise clinical benefit.
3. For late presenting high-risk patients with severe symptoms, their viral load may remain high, and initiation of antiviral treatment may still be worthwhile.
4. More stringent infection control measures, including strict droplet precautions and preferably isolation for an extended period of time may be necessary owing to prolonged viral shedding.
5. Randomised, controlled trials are indicated to address timing and dosage of treatment for severe influenza infection.

Hong Kong Med J 2013;19(Suppl 4):S15-8

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RFCID project number: 06060282

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Introduction

Influenza is estimated to be responsible for 4.1 to 4.5 million excess illnesses in individuals aged 20 years and older. Its symptoms are debilitating, and lower respiratory (eg bronchitis, pneumonia) and non-respiratory (eg cardiovascular, cerebrovascular) complications are common. Influenza-related complications result to approximately 150 000 hospitalisations and 20 000 deaths annually in the United States.

Viral kinetics and its clinical correlation with human influenza infections are not well known, especially in sicker, hospitalised patients who are usually elderly and have co-existing medical illnesses. In young children and immunocompromised hosts, influenza-virus titres correlated with symptom severity, and extensive virus shedding can persist for prolonged periods. We hypothesised that patients with severe, complicated influenza infection may have higher viral loads and more prolonged viral shedding. If confirmed, such patients should be subject to more aggressive antiviral treatment and more stringent infection control measures.

Methods

This prospective, observational study was conducted from January to December 2007. Ethics approval was obtained from the Institutional Review Boards of the Hospital Authority of Hong Kong and The Chinese University of Hong Kong. Consecutive laboratory-confirmed patients aged ≥16 years with influenza who were admitted to the medical department of the Prince of Wales Hospital (PWH) were recruited. All patients presenting with acute febrile respiratory illness needing hospitalisation were admitted to designated medical wards and managed according to a standard protocol. Nasopharyngeal aspiration and immunofluorescence assay was used for case identification. Patients might be prescribed a standard course of oseltamivir (Tamiflu) 75 mg twice daily for 5 days, based on existing recommendations. Patients were managed and discharged according to usual clinical practice.

Patients were recruited regardless of disease severity and treatment status after informed consent. A pair of combined nasal and throat swabs was collected from each patient starting from the day of recruitment (baseline) and then serially until week 1. All specimens were sent for viral RNA quantification (viral load) and virus isolation. Clinical data was recorded including demographics, medical comorbidities (eg congestive heart failure, cerebrovascular, neoplastic, chronic liver and renal diseases, chronic pulmonary diseases, use of immunosuppressants, and chronic respiratory conditions), influenza vaccination status, symptom onset time, symptom severity score, complications, antiviral treatment, and clinical outcomes.

Controls were patients aged 16 to 65 years presented to the emergency department of PWH with influenza but not hospitalised and without any comorbidity/complication.

All serially collected nasal/throat swabs were subjected to influenza viral RNA quantification and virus isolation. Viral RNA quantification was performed using a real-time RT-PCR technique, targeting the M-gene of influenza A and

B. Virus isolation was performed using MDCK cells. Any cytopathic effect on the cell monolayer was observed and confirmed by immunofluoresent testing. All influenza A virus isolates were sent for sub-typing (eg H3, H1).

All viral RNA concentrations were log-transformed for statistical analysis. Linear and logistic regression analyses and multilevel (mixed-effect) models were used to assess independent factors affecting viral loads.

Results

A total of 147 hosptalised influenza A cases, 29 influenza B cases, and 19 non-hospitalised influenza A controls were recruited.

Influenza A

Influenza A virus was isolated in 128 (87.1%) of the 147 cases, and the remaining cases were both IFA and RT-PCR positive. Sub-typing was performed for 126 isolates, and all were confirmed to be the H3N2 virus (Table).

Baseline samples were collected within the first week of symptom onset; 81.6% were collected on or after day 3. A median of four serial specimens was collected from each patient. Most patients were of advanced age, had comorbid illnesses, and developed influenza-related complications necessitating prolonged hospitalisation.

For the baseline samples, 88 were collected pre-treatment and 59 after one to two doses of oseltamivir. Of which, 120 (81.6%) were detectable or tested positive for influenza A virus by RT-PCR. Viral concentrations correlated positively with the four-point symptom score (Spearman's ϱ=0.219, P=0.010), and were 1.5 logs higher in hospitalised patients than in controls (5.96±1.19 vs 4.41±1.91 $\log_{10}$copies/mL, P=0.003).

The mean viral concentration of all pre-treatment samples was 4.68 (standard deviation [SD], 2.06) $\log_{10}$copies/mL. Viral concentrations correlated negatively with days from symptom onset, indicating natural viral clearance (Spearman's ϱ=-0.388, P<0.0001). Univariate analyses showed that patients with major systemic comorbidities had significantly higher baseline viral concentrations. Viral concentrations on/beyond day 3 of the illness were 1.4 logs higher in these patients than in controls (5.06±1.85 vs 3.62±2.13, P=0.005, Fig 1). Multiple linear regression analyses showed that presence of major systemic comorbidities (β=0.765, standard error [SE]=0.354, 95% confidence interval [CI]=0.065-1.465, P=0.032), longer time elapsed from symptom onset (β=-0.457, SE=0.107, 95% CI=-0.668 to -0.246, P<0.0001), and antiviral initiation (β=-0.899, SE=0.351, 95% CI=-1.592 to -0.206, P=0.011) were independent factors associated with virus concentrations, after adjusting for age and gender. Factors associated with an undetectable viral load included antiviral initiation (odds ratio [OR]=2.94, 95% CI=1.20-7.25, P=0.019) and time elapsed from symptom onset (OR=1.48, 95% CI=1.13-1.94, P=0.004), after adjusting for baseline characteristics.

The mean±SD virus concentrations on days 1, 2, 3, 4, 5, 6, and 7 of symptom onset were 6.30±1.38, 5.77±1.06, 4.30±1.87, 3.50±2.11, 2.98±2.31, 2.39±2.08, and 1.76±2.25 $\log_{10}$copies/mL, respectively. By days 4, 5, 6, and 7 of symptom onset, 78.6%, 68.5%, 52.6%, and 32.7% of these hospitalised patients still had detectable influenza viral RNA, respectively. Viral concentrations declined non-linearly with time elapsed from symptom onset (P<0.001, linear and quadratic trend likelihood ratio tests). In a final multilevel model, patients with major systemic comorbidities had persistently higher viral concentrations (mean=0.854, SE=0.266, P<0.0001, likelihood ratio test), whereas those starting antiviral treatment on day 1 (mean=

Table. Clinical characteristics and outcomes of patients with influenza A infection (n=147)

Patient characteristics	No. (%) of patients
Age >65 years	111 (75.5)
Male	77 (52.4)
Elderly home residents	22 (15.0)
Influenza vaccination*	32 (21.8)
Comorbidity†	
Any	94 (63.9)
Major systemic (congestive heart failure, cerebrovascular, neoplastic, chronic liver and renal diseases, and immunosuppression)	53 (36.1)
Influenza-related complication‡	
Any	118 (80.3)
Cardiorespiratory	104 (70.7)
Use of supplemental oxygen	88 (59.9)
Use of antiviral treatment§	110 (74.8)
Death	2 (1.4)
Transferred to convalescent care facilities	34 (23.1)
Total length of hospital stay >7 days	62 (42.2)

* Vaccination status is unknown in 14 patients

† Classification is based on the Pneumonia PORT Severity Index scoring system. 'Any' referred to the presence of major or other significant medical illnesses, including diabetes, ischemic heart disease, chronic pulmonary (asthma, chronic obstructive pulmonary disease, bronchiectasis), and neurological diseases

‡ 'Any' was defined as new or exacerbation of underlying medical problems, whereas 'cardiorespiratory' referred to pneumonia, bronchitis, exacerbation of chronic pulmonary diseases, and acute cardio-/cerebro-vascular events. Patients might have >1 complications

§ Oseltamivir is prescribed on days 1-3 (n=85) or days 4-6 (n=25). The median (interquartile range) was day 3 (2-3). No antiviral treatment was given to 37 patients

-1.301, SE=0.459, P<0.001) and on days 2-3 (mean= -0.743, SE=0.341, P=0.030) had faster declines in viral concentrations, after adjusting for baseline characteristics (Fig 2). In patients with major systemic comorbidities, virus was persistently detectable by the end of the first week in 87.5% of patients not given oseltamivir treatment, 27.3% of those received it on days 1-3, and 60.0% of those received it on days 4-6 (chi-square, P=0.011).

Overall, by days 4, 5, 6, and 7 of symptom onset, 17.2%, 8.9%, 4.2%, and 2.1% of the patients remained culture positive, respectively, as did 38.5% and 11.1% of 'untreated' and 'treated' patients, and 23.7% and 5.0% of patients with and without comorbidity. The presence of comorbidities (OR=6.98, 95% CI=1.44-33.78, P=0.016) and lack of antiviral treatment (OR=0.17, 95% CI=0.06-0.52, P=0.002) were independent factors associated with persistence of virus. Patients with major systemic comorbidities had a more prolonged (>7 days) illness and hospitalisation (56.6% vs 34.0%, P=0.008).

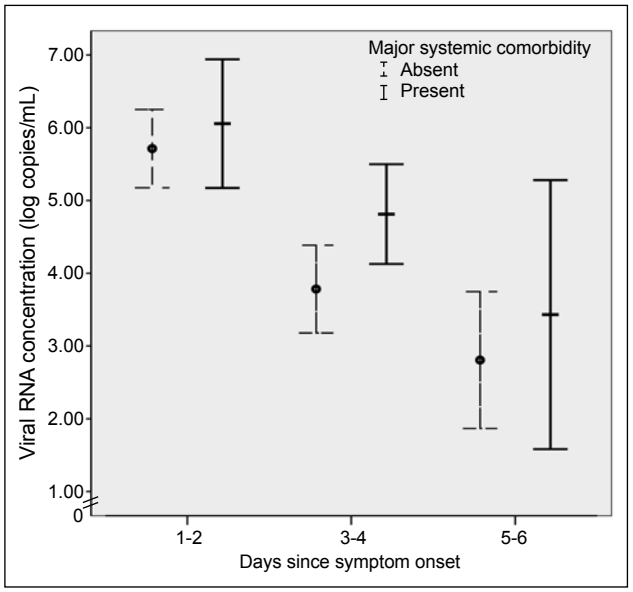

Fig 1. Baseline influenza A viral RNA concentrations according to day of symptom, in the presence or absence of major systemic comorbidities (congestive heart failure, cerebrovascular, neoplastic, chronic liver and renal diseases, and immunosuppression)

Influenza B

In 29 influenza B patients, multiple linear regression analyses of baseline samples showed that presence of comorbidities (β=2.291, SE=0.927, 95% CI=0.386-4.196, P=0.020) and longer time elapsing from symptom onset (β=-0.886, SE=0.275, 95% CI=-1.451 to -0.321, P=0.003) were independently associated with increased and decreased virus concentrations, respectively. In 69.6%, viral RNA remained detectable by the end of the first week of illness. This was associated with older age (>65 years) [92.3% vs 40.0%, P=0.019], presence of comorbidities (75.0% vs 33.3%, P>0.05), and lack of timely antiviral treatment (78.6% vs 55.6%, P>0.05). Viral cultures remained positive by days 4 and 6 of symptom onset in 56.0% and 18.5% of the patients, respectively.

Factors affecting viral clearance and outcomes

Among all cases, influenza B (OR=4.72, 95% CI=1.74-12.84, P=0.002), major systemic comorbidity (OR=2.30, 95% CI=0.95-5.56, P=0.065), and no early antiviral treatment (OR=4.95, 95% CI=2.02-12.16, P<0.001) were associated with persistence of virus. Influenza B (OR=4.45, 95% CI=1.28-15.50, P=0.019), age older than 65 years (OR=13.89, 95% CI=2.70-71.36, P=0.002), and persistently detectable viral RNA by the end of the first

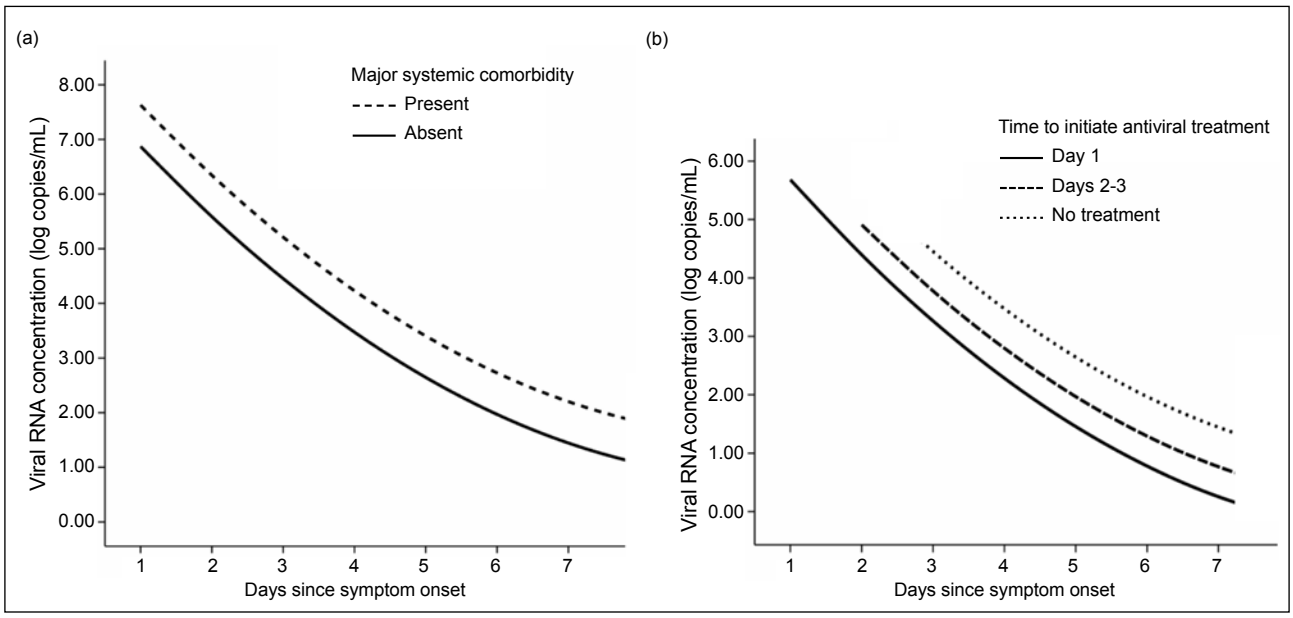

Fig 2. (a) Effects of comorbidity and (b) effects of time of treatment initiation on serial influenza A viral loads in a final multilevel model

week of illness (OR=3.42, 95% CI=1.35-8.66, P=0.009) were associated with prolonged hospitalisation and needing of convalescence care.

Discussion

In our hospitalised patients, higher viral loads correlated with more severe symptoms. Immunosuppressed patients may develop very severe influenza infection, have high viral loads and prolonged viral shedding. Elderly with underlying major systemic medical conditions (congestive heart failure, cerebrovascular, neoplastic, chronic liver and renal diseases) have more active viral replication and thus more severe illness. In these patients, natural viral clearance is slow, and the high viral load persists beyond the first 2 to 3 days of the illness. They also have significantly higher viral loads even following a standard course of antiviral treatment.

Our findings have several important implications. Previous clinical trials on neuraminidase inhibitors for seasonal influenza mostly involved young patients without underlying medical conditions. Benefit of antiviral treatment initiated after beyond 2 days from symptom onset was not demonstrated, as the viral load already declined significantly during recovery. Our results suggest that in high-risk patients with severe symptoms, the viral load may remain high, and late initiation of antiviral treatment may still be worth considering. Higher dosage of oseltamivir (eg 150 mg twice daily) may be necessary to achieve a faster viral load reduction and to prevent drug resistance. In H5N1-infected patients with very active and persistent viral replication, late initiation (on days 4-5) and higher dosage of antiviral treatment may suppress viral replication. In severe seasonal influenza, survival benefits from delayed antiviral treatment have also been reported. Randomised, controlled trials are needed to resolve these issues. Early diagnosis and treatment is important for high-risk patients. RT-PCR assay is more sensitive than culture and can be considered for rapid diagnosis. Early antiviral treatment enables more rapid reduction in viral load. Over 80% of untreated patients remain PCR positive one week after illness onset, and almost 40% remain culture positive on day 4. Therefore, strict droplet precaution coupled with isolation for an extended period is recommended. Early detection, treatment, and isolation of hospitalised influenza patients can effectively prevent nosocomial spread. In contrast to influenza A patients, influenza B patients tend to have more prolonged viral shedding and clinical symptoms, despite oseltamivir treatment. This is consistent with the recent observations that oseltamivir is less effective against influenza B, possibly related to a structural difference in its viral neuraminidase. Thus, a different treatment regimen may be needed when treating influenza B patients.

Acknowledgements

This study was supported by the Research Fund for the Control of Infectious Diseases, Food and Health Bureau, Hong Kong SAR Government (#06060282).

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