MAJOR ARTICLE

Viral Loads and Duration of Viral Shedding in Adult Patients Hospitalized with Influenza

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(See the editorial commentary by Ison, on pages 485–8)

Background. The goal of this study was to characterize viral loads and factors affecting viral clearance in persons with severe influenza.

Methods. This was a 1-year prospective, observational study involving consecutive adults hospitalized with influenza. Nasal and throat swabs were collected at presentation, then daily until 1 week after symptom onset. Real-time reverse-transcriptase polymerase chain reaction to determine viral RNA concentration and virus isolation were performed. Viral RNA concentration was analyzed using multiple linear or logistic regressions or mixed-effect models.

Results. One hundred forty-seven inpatients with influenza A (H3N2) infection were studied (mean age ± standard deviation, 72 ± 16 years). Viral RNA concentration at presentation positively correlated with symptom scores and was significantly higher than that among time-matched outpatients (control subjects). Patients with major comorbidities had high viral RNA concentration even when presenting >2 days after symptom onset (mean ± standard deviation, 5.06 ± 1.85 vs 3.62 ± 2.13 $\log_{10}$ copies/mL; $P = .005$; β, +0.86 [95% confidence interval, +0.03 to +1.68]). Viral RNA concentration demonstrated a nonlinear decrease with time; 26% of oseltamivir-treated and 57% of untreated patients had RNA detected at 1 week after symptom onset. Oseltamivir started on or before symptom day 4 was independently associated with an accelerated decrease in viral RNA concentration (mean β [standard error], −1.19 [0.43] and −0.68 [0.33] $\log_{10}$ copies/mL for patients treated on day 1 and days 2–3, respectively; $P < .05$) and viral RNA clearance at 1 week (odds ratio, 0.10 [95% confidence interval, 0.03–0.35] and 0.30 [0.10–0.90] for patients treated on day 1–2 and day 3–4, respectively). Conversely, major comorbidities and systemic corticosteroid use for asthma or chronic obstructive pulmonary disease exacerbations were associated with slower viral clearance. Viral RNA clearance was associated with a shorter hospital stay (7.0 vs 13.5 days; $P = .001$).

Conclusion. Patients hospitalized with severe influenza have more active and prolonged viral replication. Weakened host defenses slow viral clearance, whereas antivirals started within the first 4 days of illness enhance viral clearance.

Seasonal influenza is responsible for >226,000 excess hospital admissions annually in the United States [1, 2]. In Asia, the burden of influenza is substantial, and the associated morbidity and mortality is comparable to that in western countries [3, 4]. Recent studies have shown that adults hospitalized with influenza tend to present to the hospital late (>40% present >48 h after symptom onset), have serious complications and prolonged courses of illness, and a high mortality rate (4%–29%) [1, 5–10]. Older patients and those with comorbidities (eg, heart, pulmonary, liver, and renal diseases and malignancy and immunosuppression) experience the worst outcomes [1, 2, 6, 7, 9–11]. However, few data exist regarding the management of these patients, because most antiviral trials have been conducted

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among younger, previously healthy individuals and in the outpatient setting [12–16]. Viral replication patterns and the efficacy of antiviral drugs have not been adequately studied for severe influenza [17–20]. This study was undertaken to characterize viral load changes in a large hospital cohort with use of a real-time reverse-transcription polymerase chain reaction (RT-PCR) assay [21, 22] and to examine factors influencing the rate of viral RNA decrease or clearance in these patients.

METHODS

Patients and Study Design

A prospective, observational study of patients (aged >16 years) with laboratory-confirmed influenza who were admitted consecutively to the Prince of Wales hospital (Hong Kong) from 1 January through 31 December 2007 was conducted [23]. Prince of Wales hospital is a 1400-bed teaching hospital serving an urban population of 1.5 million persons in the eastern New Territories region of Hong Kong. It is operated by the Hospital Authority, the key provider of acute medical services in Hong Kong.

Patients hospitalized with influenza were diagnosed and treated according to a standard protocol [7, 8]. In brief, all adult patients presenting with acute febrile respiratory illness requiring hospitalization were admitted to designated medical wards, and droplet precautions were implemented. Patients were admitted to the hospital if they developed potentially serious medical conditions or if the exacerbation of their underlying chronic illnesses or severe symptoms were considered to be unmanageable at home [7]. Nasopharyngeal aspiration was performed for an immunofluorescence assay to detect influenza A and B infections [24–26]. According to local guidelines [1, 7, 12, 16, 25], all patients hospitalized with confirmed influenza within 2 days after symptom onset received a standard course of oseltamivir treatment (75 mg twice daily for 5 days). However, for patients who present later, the decision regarding antiviral treatment was made by their physicians. Patients were otherwise treated and discharged according to usual clinical practice [7].

Consecutive patients were recruited into the study once their diagnosis of influenza A or B was established by nasopharyngeal aspiration-immunofluorescence assay. After obtaining written informed consent, separate nasal and throat swabs were collected and combined for viral load testing. Designated research nurses were responsible for the collection of swabs by standard techniques [7, 21, 24, 26]. Specimen collection was started from the day of recruitment and continued daily until 1 week after symptom onset [18]. For patients who were discharged early, specimen collection was arranged on an outpatient basis. All swabs were sent for both viral RNA concentration determination and virus isolation.

Patients who presented to the emergency department of Prince of Wales hospital with influenza that did not require hospitalization were recruited as control subjects. Similarly, nasopharyngeal aspiration and nasal and throat swabs were obtained at presentation. Serial sampling was not performed. Patients who had positive nasopharyngeal aspiration–immunofluorescence assay results, were aged 16–65 years, and had neither documented medical comorbidity nor evidence of influenza-related complication were included for comparison. None had received antiviral treatment.

Clinical data were recorded prospectively with a standard instrument that included demographic characteristics, medical comorbidities [1, 7, 8, 10, 11, 18], influenza vaccination status, symptom/fever onset time (defined as symptom day 1), symptom severity score (recorded on a 4-point scale with regard to nasal obstruction, sore throat, cough, myalgia, fatigue, headache, and feverishness) [14, 15], influenza-related complications [7, 10, 11, 15], details of antiviral treatment received, systemic corticosteroid use for treatment of exacerbations of chronic obstructive pulmonary disease or asthma, and the clinical outcomes. Major comorbidity was defined as the presence of $\geqslant$1 of the following chronic, systemic medical conditions: congestive heart failure; cerebrovascular, neoplastic, chronic liver, or renal diseases; and use of immunosuppressants, which are associate with impaired host immune defense [1, 6–8, 10, 11, 18].

Ethical approval was obtained from the Institutional Review Boards of the Hospital Authority of Hong Kong and The Chinese University of Hong Kong. The study was supported by a government research grant.

Virological Investigations

Nasopharyngeal aspirates were subjected to immunofluorescence staining, virus isolation, and subsequent virus subtyping as described elsewhere [7, 8]. Influenza A virus isolates were differentiated into H1 and H3 subtypes by the National Influenza Reference Laboratory at the Centre for Health Protection in Hong Kong.

The nasal and throat swabs were put together into prepared bottles that contained a fixed amount (2.0 mL) of viral transport medium [21]. All samples were aliquoted into 2 equal parts. One portion was used for influenza virus isolation with Madin-Darby canine kidney cells as described in Appendix A, which appears only in the online version of the *Journal*, and elsewhere [7, 8]. Another portion was stored at −70°C for subsequent viral RNA concentration determination. All virological investigations were performed in a Biosafety Level II laboratory.

Viral RNA concentration assay. Influenza RNA concentration in nasal and throat swab samples was determined using a real-time quantitative RT-PCR method as described in Appendix A, which appears only in the online version of the

Journal, and elsewhere [7, 8]. After viral RNA extraction, the resulting complementary DNA products (cDNAs) were used immediately for real-time PCR, with primers designed to detect the influenza A (all human subtypes, including the circulating H3N2 and H1N1) or B virus RNA by targeting the M-gene [22]. The influenza virus A– and B–specific fluorogenic probes (Appendix A, which appears only in the online version of the *Journal*) were designed to anneal to an internal sequence of the amplified region [22]. The real-time PCR reactions were carried out using the StepOne real-time PCR machine (Applied Biosystems). Plasmids containing a known copy number of amplification targets were included in the real-time PCR assay to generate a standard curve for quantification of test samples. The lower detection limit for this influenza A real-time PCR assay is 250 copies/mL, and the lower limit of detection is 100 copies/mL for the influenza B real-time PCR assay.

Statistical analysis. Influenza viral RNA concentrations (expressed in copies/mL) were log transformed for statistical analyses. The student's *t*, Mann-Whitney *U*, and χ^2 tests were used for univariate comparisons when appropriate, and Spearman's rank correlation coefficient was used to assess correlations between viral concentration and clinical variables. Variables with *P* values <.1 in the univariate analyses were entered into multiple linear regression models (backward) to identify independent factors affecting initial viral concentration. Similarly, significant variables ($P < .1$) were entered into backward stepwise logistic regression models to determine independent factors associated with viral clearance (ie, undetectable viral RNA, below the detection limit of the RT-PCR assay) at 1 week after symptom onset [18]. Adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated for each explanatory variable. Multivariate Cox proportional hazards model analysis was used to determine independent factors associated with hospital discharge [7]. An adjusted hazard ratio >1 indicated a higher chance of discharge from the hospital. In all analyses, a *P* value of <.05 was considered to indicate statistical significance. All probabilities were 2-tailed. Statistical analysis was performed using SPSS software, version 14.0 (SPSS).

For longitudinal data, mixed-effects models [27] were used to assess the associations between viral concentration changes and time (days) elapsed from symptom onset, age, sex, comorbidity, initiation time of antiviral treatment, and corticosteroid use. Mixed-effect models are random effects models that take into account the hierarchical nature of the data and the within- and between-subject heterogeneity [27]. For longitudinal data, such models allow for measurements made at unequal intervals and with a varied number of measurements (ie, subjects who may have 1 or several measurements). The models are fitted by the method of restricted iterative generalized least-squares algorithm of MLn for Windows software package, version 2.10 (Institute of Education, University of London). The likelihood ratio test is used to assess the statistical significance of the estimates at the 5% level.

RESULTS

Influenza A

Patient descriptions. One hundred forty-seven patients with influenza A were included (table 1). All available virus isolates were confirmed to be the H3N2 subtype, which was the pre-

Table 1. Baseline Characteristics and Clinical Outcomes of 147 Patients Hospitalized with Influenza A Infection

Characteristic	No. (%) of patients
Aged >65 years	111 (75.5)
Nursing home resident	22 (15.0)
Male sex	77 (52.4)
Comorbidity	
Any[a]	94 (63.9)
Major[a]	53 (36.1)
Influenza vaccination[b]	32 (21.8)
Influenza-related complication[c]	
Any	118 (80.3)
Cardiorespiratory	104 (70.7)
Supplemental oxygen required	88 (59.9)
Corticosteroid use[d]	37 (25.2)
Antiviral treatment received	110 (74.8)
Death	2 (1.4)
Transferred to convalescent care facilities	34 (23.1)
Total duration of hospital stay >7 days	62 (42.2)

NOTE. All 147 patients had positive nasopharyngeal aspiration and immunofluorescence assay results. Influenza A virus was isolated from 128 (87.1%) of 147 patients; the remaining patients had positive results for both immunofluorescence assay and reverse-transcriptase polymerase chain reaction. Subtyping was performed on 126 isolates; all were confirmed to be H3N2 virus. Most patients (95.2%) provided ⩾2 serial samples; the remainder did not return for subsequent sampling after discharge ($n = 6$) or death ($n = 1$). Twenty-five inpatients with confirmed influenza infection refused consent for the study.

[a] Medical comorbidity (major) classification is based on the Pneumonia PORT Severity Index scoring system [7, 8]. Major, systemic comorbidities include congestive heart failure; cerebrovascular, neoplastic, chronic liver, and renal diseases; and use of immunosuppressants. These patients might also have other coexisting illnesses [10, 18]. Comorbidity (any) refers to the presence of major, systemic, or other significant medical illnesses, including mainly chronic lung diseases (asthma, chronic obstructive pulmonary disease, or bronchiectasis; $n = 35$), diabetes, ischemic heart disease, and neurological diseases [7, 10, 18]. Only a few patients in our cohort were receiving immunosuppressants.

[b] Influenza vaccine was received prior to the influenza seasons in year 2007 (ie, October–December 2006). Vaccination status was unknown for 14 patients.

[c] Influenza-related complications (any) were defined as new or exacerbation of underlying medical problems [7, 8, 11]. Complication (cardiorespiratory) referred to pneumonia ($n = 54$), bronchitis ($n = 14$), exacerbation of chronic pulmonary diseases ($n = 27$), and acute cardiovascular/cerebrovascular events ($n = 8$) [3, 4, 7, 10, 11]. Patients might have >1 complications. Only 3 patients had confirmed secondary bacterial pneumonia (*Streptococcus pneumonia,* $n = 1$; *Haemophilus influenzae,* $n = 2$).

[d] Corticosteroid therapy refers to intravenous hydrocortisone (100 mg every 6–8 hours) or oral prednisolone (30–40 mg per day) used to treat acute exacerbation of asthma or chronic obstructive pulmonary disease (median treatment duration, 5.5 days [interquartile range, 3.3–9.8 days]).

Table 2. Initial, Pretreatment Influenza A Viral RNA Concentrations Compared Between Patients with Different Baseline Characteristics

Variables	Viral RNA concentration, mean $\log_{10}$ copies/mL ± SD	*P*
Age		
>65 years	4.87 ± 2.03	.077
≤65 years	3.89 ± 2.07	
Sex		
Male	4.27 ± 2.19	.091
Female	5.02 ± 1.91	
Comorbidity, major		
Yes	5.27 ± 1.79	.021
No	4.26 ± 2.16	
Underlying chronic lung disease		
Yes	4.71 ±1.68	0.941
No	4.67 ± 2.16	
Influenza vaccination[a]		
Yes	5.18 ±1.69	.117
No	4.38 ± 2.09	

NOTE. A total of 88 samples collected before treatment initiation were included in this subanalysis. Mean pretreatment viral concentrations (± standard deviation [SD]) were noted to be 6.30 ± 1.37 on symptom day 1, 5.84 ± 1.13 on day 2, 4.50 ± 1.87 on day 3, 4.54 ± 2.09 on day 4, 4.17 ± 2.45 on day 5, and 2.63 ± 2.21 $\log_{10}$ copies/mL on day 6. Refer to the footnotes of table 1 for explanation of major comorbidity and underlying chronic lung disease.

[a] Influenza vaccines were received prior to the influenza seasons in 2007 (ie, October–December 2006). Vaccine recipients were significantly older and more frequently had major comorbidities.

dominant circulating strain during the period [23]. The mean (± standard deviation [SD]) age of patients was 71.8 ± 15.8 years; the majority had coexisting medical conditions (64%) and developed cardiac or respiratory complications (71%). The median duration of hospitalization was 7 days (interquartile range [IQR], 5–14 days). Two (1.4%) patients died. Altogether, 110 (75%) patients received oseltamivir treatment on diagnosis (50 started on symptom day 1–2, 51 started on symptom day 3–4, 9 started after symptom day 4); the median time of oseltamivir treatment initiation was day 3 (IQR, 2–3 days). Thirty-seven patients did not receive antiviral treatment, of which 36 received a diagnosis on symptom day 3 or after. Initial viral concentration measurement was obtained on symptom day 3 or after in 82% of cases. A median of 4 (IQR, 3–5) serial measurements were obtained from each patient. In 88 patients, the initial specimen was obtained immediately before treatment; in the remainder, the initial specimen was obtained soon after treatment initiation (ie, after 1–2 doses of oseltamivir).

Analyses of initial viral concentrations. Initial viral concentration among hospitalized patients ($n = 147$) was shown to correlate positively with the simultaneous 4-point symptom scores (Spearman's ρ, +0.219; $P = .010$). Initial, pretreatment viral concentration was also compared between hospitalized patients with influenza and time-matched, outpatient controls (who presented within 2 days of illness onset). The outpatients were <65 years of age and had no underlying comorbidities; none had received antiviral treatment. We found that the mean viral concentration (±SD) among hospitalized patients ($n = 22$) was higher than that among outpatients ($n = 16$) by >1.4 $\log_{10}$ copies/mL (5.96 ± 1.19 vs 4.51 ± 1.67 $\log_{10}$ copies/mL; $P = .003$].

Factors affecting viral load before starting treatment are shown in table 2. Patients with major (systemic) comorbidities had higher initial viral concentrations. Among patients who presented beyond 2 days after symptom onset, viral concentration (±SD) was also found to be significantly higher in those with major comorbidities than in those without (5.06 ± 1.85 vs 3.62 ± 2.13 $\log_{10}$ copies/mL, $P = .005$) (figure 1). There was a negative correlation between viral concentration and time elapsed from symptom onset, indicating spontaneous decrease (Spearman's ρ, −0.388; $P < .001$). Using multiple linear regression analysis, major comorbidities (β, +0.858; standard error [SE], 0.415; 95% CI, +0.032 to +1.683; $P = .042$), and a longer time lapse after symptom onset (β, −0.328; SE, 0.114; 95% CI, −0.555 to −0.101; $P = .005$) were shown to be in-

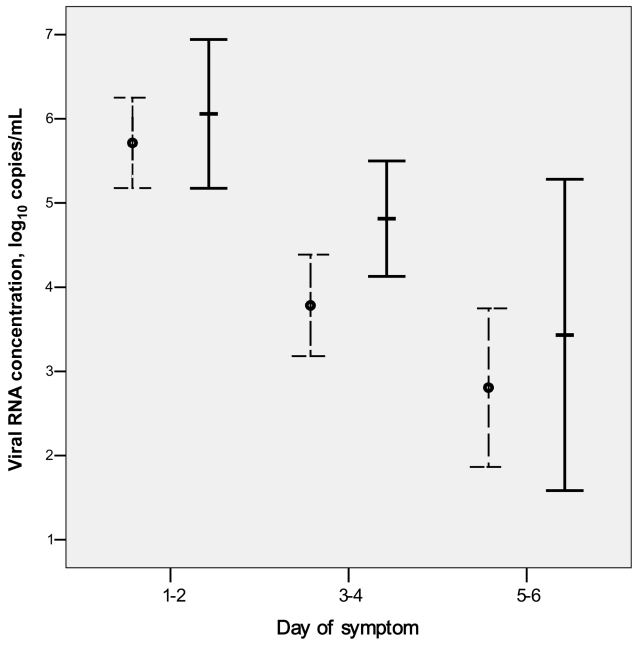

Figure 1. Influenza A viral RNA concentration at time of presentation shown according to day of symptom in patients with (*solid lines*) or without (*hatched lines*) major comorbidities. Error bars represent the standard error of the mean. Major comorbidity refers to chronic systemic medical illnesses, including congestive heart failure; cerebrovascular, neoplastic, chronic liver and renal diseases; and use of immunosuppressants (table 1).

Table 3. Explanatory Variables in a Final Multiple Linear Regression Model for Initial Influenza A Viral Concentrations ($n = 147$)

Variable affecting viral concentration	β (95% CI)	SE	P
Day from symptom onset (continuous)	−0.457 (−0.668 to −0.246)	0.107	<.001
Comorbidity, major (yes vs no)	+0.765 (+0.065 to +1.465)	0.354	.032
Antiviral initiated (yes vs no)	−0.899 (−1.592 to −0.206)	0.351	.011

NOTE. Age and sex were adjusted in the model. For 88 patients, the initial specimen was taken immediately before treatment started (antiviral initiated, no); for 59 patients, the initial specimen was taken after 1–2 doses of oseltamivir treatment had been received (antiviral initiated, yes). SE, standard error; CI, confidence interval.

dependent factors associated with increased and decreased viral concentration, respectively, adjusted for age and sex.

In a final multivariate model including all initial viral concentration measurements ($n = 147$), the presence of major comorbidities, a longer time lapse after symptom onset, and antiviral treatment initiated on presentation were found to be independent factors affecting viral concentration (table 3).

Analyses of longitudinal viral concentration changes. Factors affecting longitudinal viral concentration changes were analyzed in mixed-effect models ($n = 147$). Viral concentration showed a nonlinear decrease with time ($P < .001$, by both linear and quadratic trend likelihood ratio test). Presence of major comorbidities (mean β, +0.810; SE, 0.254; $P = .001$, by likelihood-ratio test) and systemic corticosteroid use for acute exacerbations of asthma or chronic obstructive pulmonary disease (mean β, +0.602; SE, 0.295; $P = .041$) slowed viral concentration decrease; whereas antiviral treatment started on symptom day 1 (mean β, −1.189; SE, 0.430; $P = .006$) or on symptom days 2–3 (mean β, −0.676; SE, 0.326; $P = .038$) was associated with accelerated viral concentration decrease, compared with no treatment in a final model (figure 2). No interaction effect was found between these variables.

Duration of viral shedding. Prolonged viral RNA detection was observed among the hospitalized patients with influenza. Overall, by symptom day 4, 5, and 7, 103 (78.6%) of 131, 85 (68.5%) of 124, and 32 (32.7%) of 98 patients, respectively, had detectable influenza virus RNA by RT-PCR (table 4). Among patients who had not received antiviral treatment, 84.4% and 57.1% had detectable RNA by day 5 and day 7, respectively, and the proportions were even higher (>88%) among those with major comorbidities. Advanced age, major comorbidities, and systemic corticosteroid administration were significantly associated with prolonged viral RNA detection, whereas antiviral treatment started on symptom days 1–4 was significantly associated with shortened duration of viral RNA detection (RNA detected at 1 week, 25.7% vs 57.1%; $P = .007$) (table 5). A shorter time to discharge from the hospital was associated with undetectable viral RNA on day 5 (median length of stay, 6.0 vs 8.0 days; $P = .038$) and day 7 (median length of stay, 7.0 days vs 13.5 days; $P = .001$). In a Cox-proportional hazards model, undetectable viral RNA levels within 5 days after symptom onset was independently associated with hospital discharge (adjusted hazard ratio, 1.98; 95% CI, 1.34 to 2.93; $P = .001$), adjusted for age, comorbidity, and presence of complications. Results of virus isolation are also shown in table 5. Among untreated patients, 38.5% and 21.2% remained culture positive at symptom day ⩾4 and day ⩾5, respectively; among patients with comorbidities, the proportions were even higher (41.7% and 33.3%, respectively). Antiviral treatment was significantly associated with lower rates of positive culture (culture positive at ⩾4 days, 10.2% vs 38.5%; $P = .002$; and culture positive at ⩾5 days, 4.2% vs 21.2%; $P = .006$); the reduction was >25% when observed among patients with comorbidities (culture positive at ⩾5 days, 5.9% vs 33.3%; $P = .022$).

Influenza B

A total of 29 patients received a diagnosis of influenza B during the study period [23]. As with influenza A, patients with co-

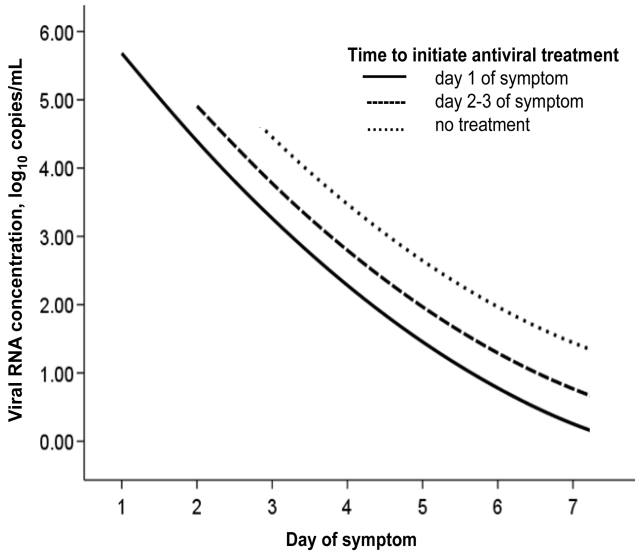

Figure 2. Effects of time of antiviral initiation on longitudinal viral load changes in a final mixed-effect model. Effects of age, sex, comorbidity, and corticosteroid use were adjusted in this final model.

Table 4. Correlation between Reverse-Transcriptase Polymerase Chain Reaction and Virus Culture Results

This table is available in its entirety in the online version of the *Journal of Infectious Diseases*

morbidities had a higher viral concentration at presentation (5.67 ± 2.45 vs 3.17 ± 1.80 $\log_{10}$ copies/mL; $P = .029$; multiple linear regression β, +2.291; SE, 0.927; 95% CI, +0.386 to +4.196; $P = .020$) (for other results, see Appendix B, which appears only in the online version of the *Journal*). Nearly 70% of patients with influenza B had detectable levels of virus by RT-PCR at 1 week of illness, and 56% remained culture positive at symptom day ⩾4 (table 5). It is notable that only 1.6% of all influenza B specimens had RT-PCR values between 100–250 copies/mL (see Methods). Mixed-effect model analysis was not performed because of the small sample size.

Factors Associated with Viral Clearance

Using multiple logistic regression models, factors associated with viral clearance (defined as negative results for viral RNA detection at day 7) were analyzed for both influenza A and B infections (table 6). It was found that antiviral treatment started on symptom days 1–4 was independently associated with early viral clearance, whereas influenza B infection, advanced age, presence of major comorbidities, and systemic corticosteroid administration were factors associated with persistent viral RNA detection. Similar factors were identified to affect persistent virus isolation at symptom day ⩾4 and day ⩾5 (Appendix C, which appears only in the online version of the *Journal*). Antiviral treatment started on symptom days 1–4 was associated with a shorter period of virus isolation, whereas influenza B infection, comorbidities, and corticosteroid administration were associated with a longer duration of persistent virus isolation.

DISCUSSION

Advanced age and presence of comorbidities are known to be associated with prolonged illness and poor outcomes in patients hospitalized with influenza infection [1, 6, 7, 9]. This study demonstrates that these patients may have higher initial viral loads and that active viral replication tends to continue beyond the first 2 days of illness, in contrast to that in healthier individuals [12–15, 17, 19]. Consistent with recent reports [18, 28], prolonged viral RNA detection (for 5–7 days after illness

Table 5. Factors Associated with Persistent Viral RNA Detection at 1 Week and Persistent Virus Isolation after 4 Days of Illness, in Patients Hospitalized with Influenza A Infection

Variable	Patients with viral RNA detected at symptom day 7, %	*P*	Patients with virus isolated on symptom day ⩾4, %	*P*
Influenza virus				
A	32.7	.001	17.2	<.001
B	69.6		56.0	
Age				
>65 years	39.0	.011	17.0	.921
⩽65 years	9.5		17.9	
Comorbidity, major				
Yes	45.7	.040	22.7	.221
No	25.4		13.9	
Systemic corticosteroid use				
Yes	53.8	.007	24.1	.256
No	25.0		14.9	
Oseltamivir initiation time				
Day 1–2	14.3	.004	2.3	<.001
Day 3–4	35.3		18.2	
Not received	57.1		38.5	

NOTE. Refer to the footnotes of table 1 for an explanation of clinical variables. Reverse-transcriptase polymerase chain reaction (RT-PCR) and virus isolation were performed for all nasal and throat swabs samples. A total of 98 patients who had viral shedding data available by 1 week after symptom onset were included in the analysis. Considering only patients with major comorbidities, persistent viral RNA detection at day 7 was noted in 87.5%, 20.0%, and 41.2% of those who received no antiviral treatment, treatment initiated on day 1–2, and treatment initiated on day 3–4, respectively ($P = .015$, by χ^2); persistent virus isolation ⩾4 days was observed in 41.7%, 0.0%, and 23.8% respectively ($P = .058$, by χ^2). Similar to other studies [18,38], the rate of positive results for culture is noted to be lower than that for RT-PCR. A reduced viral load may affect culture positivity. In all culture-positive nasal and throat swab specimens, viral RNA was successfully amplified and the viral concentration was measured.

Table 6. Factors Associated with Viral Clearance at 1 Week After Illness Onset in a Final Multiple Logistic Regression Model

Variable associated with viral clearance	Adjusted OR (95% CI)	*P*
Age >65 years (vs ≤65 years)	5.87 (1.32–26.00)	.020
Comorbidity, major (yes vs no)	2.78 (1.03–7.48)	.043
Influenza B (vs influenza A)	5.83 (1.30–26.10)	.021
Systemic corticosteroid (yes vs no)	5.44 (1.86–15.89)	.002
Oseltamivir started on symptom day 1–2[a]	0.10 (0.03–0.35)	<.001
Oseltamivir started on symptom day 3–4[a]	0.30 (0.10–0.90)	.031

NOTE. Refer to the footnotes of table 1 for an explanation of clinical variables. CI, confidence interval; OR, odds ratio.

[a] Versus no antiviral treatment within 4 days.

onset) is observed among these patients and is associated with longer duration of illness and hospitalization [7, 17–20]. Our findings suggest that prolonged viral replication may be more common than previously recognized in patients hospitalized with influenza and is not limited to immunosuppressed patients, such as those with hematological malignancies [29–32].

We found that the presence of comorbidities or concomitant systemic corticosteroid administration may slow viral clearance [33], whereas oseltamivir treatment started within the first 4 days of illness may enhance viral clearance in these patients. Oseltamivir starts to reduce the viral load after 1 day of treatment [14, 15, 17]. However viral level decrease is influenced by the timing of initiation, with the best effect (lowest viral loads) being observed with treatment initiated on the same day as symptom onset [34]. Also, different influenza virus types may show a different degree of response to oseltamivir.

There are several important implications. First, early diagnosis and therapeutic intervention for severe influenza should be emphasized [1, 7, 12, 16, 17, 21, 34]. Patients should be encouraged to present as soon as symptoms develop, despite vaccination status [7]. Prompt laboratory diagnostic testing may be used to guide treatment [1, 7, 24, 26, 35–37]; RT-PCR, if available, is the test of choice because of its high sensitivity and specificity [26, 38]. If the more sensitive tests are unavailable, empirical antiviral therapy may be considered, especially during seasonal peaks (when there is a high likelihood of influenza) and when patients are seriously ill [38]. Second, an extended therapeutic time window for initiation of antiviral therapy may be considered. Previous clinical trials involving otherwise healthy and younger, nonhospitalized patients have suggested that, to produce clinical benefits, antiviral treatment has to be initiated within 48 h after symptoms initiation, because active viral replication will have largely subsided beyond that time (determined on the basis of viral cultures) [12–17, 34]. However, among older patients with comorbidities who present after 48 h, particularly in association with severe, persistent symptoms, the viral load may remain very high (and culture positive). Recent observational studies suggested that antiviral treatment started within 4 days after illness onset might reduce mortality among adult patients hospitalized with influenza [6, 9, 39]. In this study, antiviral treatment started within 4 days of illness was observed to enhance viral load decrease and clearance when compared with no treatment, after adjustment for confounding variables [17]. Viral clearance correlates with symptom resolution and may be associated with shortened duration of hospitalization [7, 18, 19]. Randomized, controlled trials are necessary to clarify further the clinical benefit of delayed therapy, and this study of viral kinetics provides useful information for planning (eg, therapy guided by the viral load, instead of time of symptom onset) [16, 39]. Third, a more stringent approach to infection control may be necessary to avoid nosocomial transmission, because hospitalized patients may have high viral loads and a long duration of viral shedding, as detected by both RT-PCR and culture methods [17, 18, 29, 30]. These measures include strict droplet precautions and, preferably, isolation for an extended period of time (>5 days) [1, 18, 28, 29, 40], especially for patients without antiviral treatment [13, 15, 18, 28, 29, 40, 41]. In addition, aerosol-generating procedures or devices should be used with caution [29, 40, 41].

The cell-mediated inmmune response is important in controlling influenza infection [33]. Use of systemic corticosteroids to treat concomitant medical conditions during severe influenza infection was shown to be associated with a slow viral decrease and clearance (adjusted for confounders). Corticosteroids have been observed to slow viral clearance in other serious respiratory viral infections such as in respiratory syncytial virus and severe acute respiratory syndrome–associated coronavirus infections [42, 43]. However, the clinical significance of this is unknown, and its impact on the course of disease requires further investigation in controlled studies [41, 42].

In vitro data have suggested that different influenza viruses (H3N2, H1N1, influenza B) may differ in their susceptibility toward neuraminidase inhibitors [44, 45]. Consistent with other recent reports [7, 45–47], influenza B is shown to have

a slower virological response to oseltamivir. Thus, alternative treatment regimens for severe influenza B infection, such as a higher oseltamivir dosage or another neuraminidase-inhibitor, deserve clinical evaluation [46, 47].

This study is limited by its observational design and being conducted in a single center. Patients diagnosed within 2 days after symptom onset received treatment [7, 25], but there was no formal recommendation regarding treatment of patients who presented later. However, provider bias may not explain our findings, because it is more likely that sicker patients with ongoing symptoms (which reflect active viral replication) were prescribed antiviral treatments despite their later presentation, whereas those patients with subsiding symptoms were left untreated. Also, virus concentrations in the upper respiratory tract (assayed with nasal and throat swabs) may not precisely reflect the virus replication levels in the lower respiratory tract, although this approach has been used successfully to provide a semiquantitative estimate of the virus burden in studies of natural infections [8, 14, 15, 21]. Similar to other studies, we observed lower positive rates for virus culture, compared with RT-PCR assay [18, 26, 38]. Although RT-PCR could have detected nonviable virus, it is also possible that the culture method is too insensitive to detect a lower level of virus excretion (because of treatment or natural defense; see Appendix A, which appears only in the online version of the *Journal*) or that the results are falsely negative because of virus inactivation in vitro [18, 38]. These implications on infectiousness would require further study. Nonetheless, we still observed a high proportion (>20% to >40%) of untreated patients with persistent virus isolation beyond day 4–5 of illness, which strengthened our conclusions.

On the basis of our findings, areas of further research regarding the management of patients hospitalized with influenza should include clinical trials on delayed antiviral therapy in compromised and seriously ill patients [16, 39, 48], the use of a higher-dose regimen (eg, 150 mg oseltamivir twice daily), a more prolonged course of treatment (>5 days) [14, 16, 39, 48], the risk of emergence of antiviral resistance [44, 48, 49], the role of rapid diagnostic tests in different clinical settings (test performances can be affected by viral load and specimen type) [7, 24, 26, 35–38, 50], the adequacy of currently recommended infection control measures [29, 40, 41], and viral kinetics of other influenza virus subtypes (eg, H1N1). Such information is important for influenza pandemic preparedness [1, 39, 41].

In conclusion, hospitalized adult patients with influenza may have more active and prolonged viral replication. Weakened host defense slows viral clearance, whereas antiviral treatment within the first 4 days of illness enhances viral clearance. More aggressive management and infection control approaches appear warranted in these patients.

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