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The novel 2009 influenza A(H1N1) virus has disseminated globally from Mexico and the United States since April 2009. Because of the sustained human-to-human transmission at all continents, the World Health Organization raised the pandemic alert level from phase 5 to phase 6 on June 11, 2009. Because most of the global population aged <60 years has undetectable neutralizing antibody, the disease is expected to spread throughout the next few years with a more severe second wave in winter 2009/2010. The initial animal studies suggested that pandemic A(H1N1) also replicates in the lower respiratory tract and elicits more inflammatory damage than seasonal influenza A/H1N1 viruses. Although the case-fatality rate outside Mexico was comparable to that of seasonal influenza, it was much higher in Mexico, and most occurred in those aged <60 years. Because an effective pandemic A(H1N1) vaccine was not available during the first 6 months of this pandemic, oral oseltamivir was recommended for chemoprophylaxis and treatment as containment and mitigation measures. Although cases of oseltamivir-resistant isolates in relation to the neuraminidase histidine 274-to-tyrosine mutation (H274Y) were reported, most of the viral isolates are still susceptible to oseltamivir in vitro testing. Oseltamivir treatment had been shown to be safe and reduce disease duration by up to 1.5 days and incidence of secondary...
complications, such as pneumonia or otitis media, when initiated within 36 h of symptom onset in seasonal influenza A.\textsuperscript{13,14} It can be easily administered orally and is the only safe drug in patients with asthma.\textsuperscript{15} The evidence of its effectiveness for the treatment of pandemic A(H1N1) remains uncertain. The current recommendations were based on the assumption that pandemic A(H1N1) will have similar biologic characteristics as seasonal influenza A(H1N1) viruses.\textsuperscript{19} We compared the viral load profile of the first group of pandemic A(H1N1)-infected patients with or without oseltamivir treatment in Hong Kong.

**Materials and Methods**

During the containment phase of the pandemic A(H1N1) outbreak in Hong Kong from April 26, 2009, to June 18, 2009, all patients with laboratory-confirmed pandemic A(H1N1) infection, under the local Prevention and Control of Disease Ordinance, were compulsorily isolated.\textsuperscript{16,17} Only those isolated in the three hospitals of Hong Kong were included in this study.\textsuperscript{17} The study was approved by our institutional review board. In addition to drawing blood for routine hematologic and biochemical tests, a chest radiograph was taken if clinically indicated. If nasopharyngeal specimens tested positive by reverse transcriptase-polymerase chain reaction (RT-PCR) for the influenza A matrix gene and the pandemic A(H1N1) hemagglutinin gene, but negative for H3 and seasonal H1, 5 days of oseltamivir was recommended at a dosage adjusted for age and renal function.\textsuperscript{18} Those who refused oseltamivir treatment were regarded as cases for our study of natural viral load profile from upper respiratory specimens, whereas those treated were controls. Demographic, clinical, laboratory, and radiographic data were retrospectively retrieved from the computerized Clinical Management System for entry on a standard form for analysis as previously described.\textsuperscript{19} The number of classic initial clinical symptoms as predictors of influenza infections were used to correlate with viral load in respiratory specimens.\textsuperscript{20,21} These included the presence of fever (temperature $\geq 37.8^\circ$C), cough, sore throat, nasal symptoms, myalgia, and headache.\textsuperscript{20,21}

**Specimen Collection**

Respiratory specimens of patients were taken by nasopharyngeal aspirate (NPA), nasopharyngeal swab (NPS), or naso-throat swab (NTS) and sent in viral transport medium at presentation and days 1, 3, 5, and 6 after hospitalization.

**RT-PCR**

Total nucleic acid extraction using NucliSens easyMAG instrument (bioMerieux; Durham, NC) and RT-PCR for influenza A virus matrix, the H1 of pandemic A(H1N1), and seasonal H1 and H3 genes was performed as previously described.\textsuperscript{22-25} Positive controls with a swine H1 virus (A/SW/HK/294/09), H1N1 (A/California/04/2009), and human seasonal H1N1 and H3N2 viruses were included. For quantitative assay, RT-PCR targeting matrix gene was performed with forward primer [5'-CTTCAACCCGAGTGCAGAAACG-3'] and reverse primer [5'-GGCATTTTGGAACAAKCGTCTA-3'].\textsuperscript{26} The cDNA was amplified in a Lightcycler instrument with a FastStart DNA Master SYBR Green I Mix reagent kit, and a reference standard was prepared using pCRHI-TOPO vector (Invitrogen; San Diego, CA).

**Statistical Analysis**

Non-treated pandemic A(H1N1)-infected patients and those treated with oseltamivir were compared regarding their demographics, underlying comorbidities, initial presenting symptoms, laboratory parameters, and viral load of NPA at different days post symptom onset. Viral load of NPA of nontreated and those on oseltamivir initiated $\leq 2$ and $>2$ days post symptom onset were compared. Viral load of nontreated and treated patients at each interval post symptom onset were compared. $\chi^2$ Test or Fisher exact test were used for categorical variables and independent $t$ test for continuous variables between two groups. One-way analysis of variance was used to compare viral loads of NPA, NPS, and NTS at each interval post symptom onset within nontreated and treated patients. Linear regression was used to determine the rate of viral load reduction. Pearson correlation was used to test the correlations between the age, number of presenting symptoms, concomitant total WBC count, absolute lymphocyte (lym) count, hemoglobin (Hb) level, and platelet (plt) count with viral load, respectively. SPSS 17.0 for Windows (SPSS Inc.; Chicago, IL) was used for statistical computation. A two-tailed $P$ value $<.05$ was considered significant.

**RESULTS**

One hundred forty-five patients diagnosed with pandemic A(H1N1) infection from the period since the beginning of the epidemic in Hong Kong were included. Sixty-one (42%) were male patients. Seventy-six patients (52.4%) were aged $<18$ years but none were aged $<1$ year. Twenty-seven patients (18.6%) who refused treatment were enrolled as cases and 118 (81.4%) patients who received oseltamivir were controls. Refusals were related to fear of possible side effects of oseltamivir despite counseling by attending clinicians. None had diabetes mellitus or chronic obstructive airway disease. Only two (1.4%) patients had mild pneumonic changes over right middle and lower lobes on chest radiograph, respectively. None had evidence of rhabdomyolysis, myocarditis, or encephalitis. The nontreated and treated patients were comparable in demographics, comorbidity, presenting symptoms, and initial laboratory parameters (Table 1). Oseltamivir was initiated on the same day (16/118, 13.6%), 1 day (40/118, 33.9%), 2 days (27/118, 22.9%), 3 days...
Table 1—Demographics, Initial Presenting Symptoms, and Laboratory Parameters of Treated and Nontreated Pandemic A(H1N1)-Infected Patients

| Characteristics                           | Nontreated Patients (n = 27) | Treated Patients (n = 118) | P Value |
|-------------------------------------------|-----------------------------|---------------------------|---------|
| Age, y                                     | 17.6 ± 10.2                 | 21.3 ± 11.8               | .108    |
| Sex, male:female                          | 16 (59.3%)                  | 82 (69.5%)                | .305    |
| Non-Chinese ethnicity                     | 6 (21.2%)                   | 133 (111.4%)              | .078    |
| Comorbidity                               |                             |                           |         |
| Hypertension                              | 2 (7.4%)                    | 22 (18.5%)                | .049    |
| Ischemic heart disease                    | 0 (0%)                      | 2 (1.7%)                  | .645    |
| Asthma                                    | 1 (3.7%)                    | 6 (5.1%)                  | .160    |
| Smoking                                   | 0 (0%)                      | 6 (5.1%)                  | .160    |
| Contact history                           | 17 (62.9%)                  | 50 (42.4%)                | .053    |
| Use of steroid or immunosuppressants      | 1 (3.7%)                    | 1 (8.3%)                  | .399    |

Presenting symptoms

| Symptom                  | Nontreated (n = 27) | Treated (n = 118) | P Value |
|--------------------------|---------------------|-------------------|---------|
| Fever                    | 25 (92.6%)          | 105 (90.0%)       | .738    |
| Sore throat              | 16 (59.3%)          | 82 (69.5%)        | .305    |
| Cough                    | 16 (59.3%)          | 75 (63.6%)        | .677    |
| Running nose             | 17 (63.0%)          | 77 (65.3%)        | .822    |
| Myalgia                  | 4 (14.8%)           | 25 (21.2%)        | .597    |
| Headache                 | 1 (3.7%)            | 20 (16.9%)        | .126    |
| Other symptoms           |                     |                   |         |
| Shortness of breath      | 0 (0%)              | 6 (5.1%)          | .645    |
| Nausea or vomiting       | 0 (0%)              | 4 (3.4%)          | .100    |
| Diarrhea                 | 0 (0%)              | 6 (5.1%)          | .594    |
| Ear pain                 | 0 (0%)              | 1 (8.3%)          | .399    |

Initial laboratory parameters

| Parameter                  | Nontreated (n = 27) | Treated (n = 118) | P Value |
|----------------------------|---------------------|-------------------|---------|
| WBC count, 10^9/L          | 5.33 ± 1.93         | 5.80 ± 1.8        | .407    |
| Neutrophil count, 10^9/L   | 3.02 ± 1.93         | 3.74 ± 1.89       | .191    |
| Lymphocyte count, 10^9/L   | 1.61 ± 0.76         | 1.30 ± 0.66       | .166    |
| Hb, g/dL                   | 13.8 ± 1.42         | 13.8 ± 1.3        | .984    |
| Pct count, 10^9/L          | 185 ± 32            | 204 ± 49          | .076    |
| Sodium, mmol/L             | 139 ± 3             | 139 ± 2           | .936    |
| Potassium, mmol/L          | 4.1 ± 0.5           | 3.9 ± 0.4         | .147    |
| Urea, mmol/L               | 3.32 ± 1.12         | 3.80 ± 1.3        | .195    |
| Creatinine, μmol/L         | 68 ± 19             | 74 ± 21           | .409    |
| Albumin, g/L               | 39 ± 13             | 42 ± 3.2          | .459    |
| Globulin, g/L              | 33 ± 4              | 31 ± 4            | .183    |
| Total bilirubin, μmol/L    | 10 ± 6              | 9 ± 4             | .638    |
| Alkaline phosphatase       | 119 ± 64            | 76 ± 43           | .069    |
| International Unit/L       | 10 ± 7              | 10 ± 7            | .885    |
| Alanine transaminase       | 16 ± 8              | 22 ± 16           | .061    |
| Aspartate transaminase     | 27 ± 7              | 37 ± 46           | .295    |
| Creatinine kinase          | 149 ± 105           | 128 ± 114         | .721    |

Values given are N. (%) or mean ± SD unless otherwise noted. A P value < .05 was considered statistically significant. Hb = hemoglobin; pandemic A(H1N1) = pandemic 2009 influenza A(H1N1); plt = platelet.

*χ^2* test used for statistical analysis.

>Fisher exact test used for statistical analysis.

(15/118, 12.7%), and ≥4 days (20/118, 16.9%) post symptom onset. The mean (range) interval between symptom onset and oseltamivir initiation was 2.1 (0-8) days. Six patients had nonspecific complaints of nausea, vomiting, or loose stool on the initial 2 days of oseltamivir therapy but it was difficult to differentiate from clinical symptoms of pandemic A(H1N1) infection. None reported major side effects from oseltamivir therapy.

The viral loads of NPA, NPS, or NTS at different intervals post symptom onset in nontreated patients were comparable (Fig 1A, Table 2), whereas significant differences were observed at days 2 to 3 to days 8 to 9 post symptom onset in treated patients (Fig 1B, Table 3). In treated patients, viral loads of NPA were higher than those of NPS and NTS, and significant differences were observed at day 3 to 5 post oseltamivir initiation but not at 1 day before to 1 day after oseltamivir initiation (Fig 2, Table 4). Similar findings were observed in days 3 and 4 post oseltamivir initiation in those who received oseltamivir ≥2 days post symptom onset, and day 2 post oseltamivir initiation in those who received oseltamivir >2 days post symptom onset. When only NPA specimens are analyzed, viral load of NPA was significantly lower in treated than nontreated patients at day 5 post symptom onset irrespective of the relation between oseltamivir initiation and the day post symptom onset (Fig 3A, Table 5).

When oseltamivir was initiated ≤2 days post symptom onset, a greater rate of viral load reduction in NPA of treated patients (−0.638 [95% CI, −0.809 to −0.466]) vs −0.409 [95% CI, −0.663 to −0.185]) log10 copies/mL/d post symptom onset) than that of nontreated patients was observed. Similar rate of viral load reduction in NPA was observed in those who received oseltamivir ≤2 and ≥2 days of symptom onset (−0.711 [95% CI, −1.057 to −0.366]) vs −0.695 [95% CI, −0.892 to −0.549] log10 copies/mL/d post oseltamivir initiation). At day 6 post oseltamivir initiation, >90% of these patients had undetectable viral load level in respiratory specimens (Fig 3B) and their viral load level of NPA was undetectable, which was 1 day earlier than those received oseltamivir >2 days post symptom onset. Moreover, the viral load of NPA in those who received oseltamivir ≤2 days post symptom onset was consistently lower than that of nontreated patients, and significant differences were observed at days 4 to 5 and days 6 to 7 post symptom onset (Fig 4, Table 6).

Among the 385 viral load tests performed, 19 out of 73 samples from 13 nontreated patients and 133 out of 312 samples from 96 treated controls had concomitant peripheral blood taken for hematologic test. The viral load level was inversely correlated with concomitant lym count (Pearson r = −0.365, P < .001), Hb level (Pearson r = −0.234, P = .008), and plt count (Pearson r = −0.207, P = .019) in treated patients.
Antiviral therapy for influenza is hampered by the early peaking of the viral load, which leaves a narrow window of opportunity for antiviral treatment.\textsuperscript{14,24} In both seasonal and pandemic A(H1N1) influenza A infection, the viral loads peaked at around 1 to 2 days post symptom onset in natural infections of healthy adults with or without oseltamivir treatment.\textsuperscript{14,24} As no data has yet been reported on the treatment effectiveness of oseltamivir on pandemic A(H1N1) infections, it is reassuring to observe that treated patients had a faster resolution of fever (temperature \(\leq 37.2^\circ{\text{C}}\)) as compared to nontreated patients (1.4 vs 2.8 days, \(P = .012\)). None required ventilatory or critical care and no death was reported.

Similar findings were obtained with lymphocyte count (Pearson \(r = -0.687, \ P = .001\)) and WBC counts (Pearson \(r = -0.489, \ P = .034\)), but not for Hb level and plt count of nontreated patients. No significant correlation was observed between the viral load level with patient’s age and the number of classic presenting symptoms. Resolution of fever (temperature \(\leq 37.2^\circ{\text{C}}\)) was 1.4 days earlier in treated than in nontreated patients (1.4 vs 2.8 days, \(P = .012\)). None required ventilatory or critical care and no death was reported.

\textbf{FIGURE 1.} (A) The mean \pm SD viral load (log\(_{10}\) copies/mL) profile in different respiratory specimens of pandemic A(H1N1)-infected patients not treated with oseltamivir at different intervals (days) post symptom onset. The detection limit of the quantitative RT-PCR was 2.95 log\(_{10}\) copies/mL. (B) The mean \pm SD viral load (log\(_{10}\) copies/mL) profile in different respiratory specimens of pandemic A(H1N1)-infected patients treated with oseltamivir at different intervals (days) post symptom onset. The detection limit of the quantitative RT-PCR was 2.95 log\(_{10}\) copies/mL. NPA = nasopharyngeal aspirate; NPS = nasopharyngeal swab; NTS = naso-throat swab; pandemic A(H1N1) = pandemic 2009 influenza A(H1N1); RT-PCR = reverse transcriptase-polymerase chain reaction.

\textbf{DISCUSSION}
had a greater rate of viral suppression in respiratory specimens throughout the disease course, with significantly lower viral load at day 5 post symptom onset. Moreover, when oseltamivir was initiated ≤2 days of symptom onset, viral load was not detectable at day 6 post oseltamivir, which was 1 day after treatment completion, and was 1 day earlier than that of those initiated >2 days of symptom onset. The effect of oseltamivir as early treatment in suppressing pandemic A(H1N1) was similar to that of seasonal influenza, although its effectiveness for pandemic A(H1N1) chemoprophylaxis was not yet established.\(^{14}\) Fever subsided 1.4 days earlier in treated patients, which is consistent with previous findings that oseltamivir shortens fever duration and reduces the quantity and duration of viral shedding in adults with seasonal influenza.\(^{14,27,28}\)

Although oseltamivir was shown to hasten recovery and reduce viral load in this study, its long-term effectiveness for pandemic A(H1N1) remains uncertain. Primary resistance to the neuraminidase inhibitors among wild strains of human influenza viruses A(H1N1), A(H3N2), and B has been uncommon until the recent few years.\(^{14}\) Oseltamivir resistance, which is largely associated with H274Y mutations in neuraminidase gene of influenza A(H1N1) and H5N1 viruses,\(^{29-31}\) developed in 0.33% to 5.5% of patients following oseltamivir treatment.\(^{14,28-32}\) In 2007, H274Y mutants of influenza A(H1N1) Brisbane-like variant, which were first detected in Norway,\(^{33}\) had spread globally and become the dominant seasonal H1N1 virus. Initial bioinformatics analysis and phenotypic antiviral susceptibility tests showed that the H1N1 is susceptible to oseltamivir and zanamivir but resistant to amantadine or rimantadine because of a serine 31-to-asparagine mutation.\(^{34}\) With the dramatic increase in oseltamivir use for treating pandemic A(H1N1) infection, resistance may continue to increase worldwide, including Hong Kong.\(^{35,36}\) Even with the availability of a safe and effective pandemic A(H1N1) vaccine, antiviral stockpiling remains an important armamentarium for the epidemiologic control of future pandemic.

The early control of viral load in patients not treated with oseltamivir may be explained by the actions of innate immunity followed by early anamnestic adaptive immune response, possibly cytotoxic T lymphocyte response against cross-reacting epitopes of internal proteins or nonneutralizing surface proteins common to all influenza A viruses.\(^{37}\) Failure to mount this early immune response because of innate humoral or cellular immunodeficiencies, concomitant use of immunosuppressive drugs, or transient immunoparesis due to severe concomitant coinfection may predispose some patients to develop severe clinical progression.\(^{38}\) They are often those who presented late with hospitalization at a median of 7 days with respiratory failure and death at a median of 10 days post symptom onset.\(^{3}\) Viral load reflects the dynamic interaction between viral replication and clearance by body defense mechanisms. Monitoring viral load throughout the disease course has been used as an objective means of checking the clinical progress or response to antiviral therapy. We showed the inverse correlation between the absolute lymphocyte count and concomitant viral load level in

### Table 2—Viral Load in Different Respiratory Specimens of Pandemic A(H1N1)-Infected Patients Not Treated With Oseltamivir at Different Intervals Post-Symptom Onset

| Respiratory Specimens | Interval Post-Symptom Onset, d | NPA | NPS | INPS | sINPS | NTS |
|-----------------------|-------------------------------|-----|-----|------|-------|-----|
|                       | 0-1                          | 2-3 | 4-5 | 6-7  | 8-9   |
| NPA                  | 8.00 ± 1.30 (7)              | 7.22 ± 0.94 (10) | 6.90 ± 1.80 (6) | 5.64 ± 1.27 (8) | 6.75 ± 0.09 (4) |
| NPS                  | 7.43 ± 0.80 (4)              | 8.57 ± 0.43 (2)  | 5.84 ± 1.81 (6) | 4.06 ± 0.95 (5) | 7.18 ± 1.73 (3)  |
| INPS                 | (1)                          | (1)  | (2)  | (4)  | (1)   |
| sINPS                | (3)                          | (1)  | (4)  | (1)  | (3)   |
| NTS                  | 7.18 ± 4.47 (6)              | 5.74 ± 1.52 (5) | 4.35 ± 1.59 (1) | 4.87 ± 0.78 (7) | 6.90 ± 0.80 (5) |
| P value              | .814                         | .183 | .164 | .079 |       |

Values given are mean ± SD viral load in log\(_{10}\) copies/mL (No. of specimens). The detection limit of the quantitative RT-PCR was 2.95 log\(_{10}\) copies/mL.

### Table 3—Viral Load in Different Respiratory Specimens of Pandemic A(H1N1)-Infected Patients Treated With Oseltamivir at Different Intervals Post-Symptom Onset

| Respiratory Specimens | Interval Post-Symptom Onset, d | NPA | NPS | INPS | sINPS | NTS |
|-----------------------|-------------------------------|-----|-----|------|-------|-----|
|                       | 0-1                          | 2-3 | 4-5 | 6-7  | 8-9   |
| NPA                  | 7.78 ± 1.66 (45)             | 7.02 ± 1.88 (27) | 5.65 ± 1.84 (21) | 5.20 ± 1.84 (15) | 3.51 ± 1.19 (9) |
| NPS                  | 7.19 ± 1.95 (11)             | 6.48 ± 2.83 (19) | 4.98 ± 1.73 (20) | 3.90 ± 1.26 (19) | 2.95 ± 0.4 (4) |
| INPS                 | (0)                          | (8)  | (12) | (14) | (0)   |
| sINPS                | (11)                         | (11) | (8)  | (5)  | (4)   |
| NTS                  | 6.75 ± 1.21 (9)              | 5.18 ± 1.78 (26) | 3.57 ± 1.21 (32) | 2.95 ± 0.70 (17) | 2.95 ± 0.12 (12) |
| P Value              | .317                         | .009 | <.001| .001 | .031  |

Values given are mean ± SD viral load in log\(_{10}\) copies/mL (No. of specimens). The detection limit of the quantitative RT-PCR was 2.95 log\(_{10}\) copies/mL. See Table 2 for expansion of abbreviations.

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aspirate is more likely to get a good sample of infected cells than swabbing the throat, anterior nares, or even the nasopharynx. As the viral load is decreased by oseltamivir, the difference would be accentuated in the suboptimal sampling methods by NPS and NTS. But these factors are unlikely to affect the overall conclusion of this study because the results were similar when only nasopharyngeal aspirate was analyzed. Similar to previous studies, oseltamivir is associated with few side effects. The most frequent side effects are gastrointestinal symptoms, which occurred in up to 40% of school children who received oseltamivir prophylaxis for the influenza A(H1N1) virus. Previous reports suggesting serious neuropsychiatric manifestations after oseltamivir were not demonstrated in later studies and only 18% of school children taking oseltamivir prophylaxis had reported mild symptoms.

Lymphopenia was noted in pandemic A(H1N1)-infected patients of greater severity, especially those who were pregnant or morbidly obese. Previous study in immunocompromised patients showed that lymphocyte recovery was associated with viral clearance, independent of oseltamivir. There was no difference in viral load with different sampling methods in nontreated patients, whereas the differences seen with NPA, NPS, and NTS in treated patients could be attributed to increased statistical power with the higher number of patient specimens in this group. As the dominant site of viral replication in these mild cases is the nasopharynx rather than the lower respiratory tract, the suction by nasopharyngeal aspirate is more likely to get a good sample of infected cells than swabbing the throat, anterior nares, or even the nasopharynx. As the viral load is decreased by oseltamivir, the difference would be accentuated in the suboptimal sampling methods by NPS and NTS. But these factors are unlikely to affect the overall conclusion of this study because the results were similar when only nasopharyngeal aspirates were analyzed.

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Table 4—Viral Load in Different Respiratory Specimens of Pandemic A(H1N1)-Infected Patients at Different Days Post-Oseltamivir Initiation

| Respiratory Specimens | No. of d Post-Oseltamivir Initiation |
|-----------------------|-------------------------------------|
|                       | 1    | 2    | 3    | 4    | 5    | 6    | 7    |
| NPA                   | 7.58 ± 1.14 | 7.69 ± 1.62 | 6.56 ± 2.55 | 6.21 ± 2.21 | 5.57 ± 1.61 | 5.32 ± 1.54 | 4.48 ± 0.96 | 3.07 ± 0.72 | 2.95 ± 0.41 |
| (18)                  | (40)  | (13) | (9)  | (10) | (7)  | (6)  | (7)  | (5)  |
| NPS                   | 7.23 ± 0.7 (1) | 7.19 ± 2.40 | 6.27 ± 2.43 | 4.33 ± 1.50 | 4.13 ± 1.70 | 5.08 ± 1.65 | 4.26 ± 1.49 | 2.95 ± 0.70 | 2.95 ± 0.78 |
| (14)                  | (13) | (3)  | (17) | (13) | (7)  | (5)  | (2)  | (2)  |
| INPS                  | (0)  | (1)  | (6)  | (3)  | (12) | (7)  | (5)  | (2)  | (2)  |
| sNPA                  | (1)  | (13) | (7)  | (0)  | (5)  | (6)  | (2)  | (0)  | (4)  |
| NTS                   | 6.76 ± 0.37 | 6.04 ± 1.36 | 5.59 ± 2.12 | 4.30 ± 1.77 | 3.48 ± 0.92 | 3.31 ± 1.06 | 2.95 ± 0.37 | 2.95 ± 0.70 | 2.95 ± 0.70 (4) |
| (2)                   | (6)  | (17) | (19) | (17) | (10) | (12) | (9)  |
| P value               | .606 | .123 | .604 | .066 | .003 | .014 | .002 | .021 | .535 |

Values given are mean ± SD viral load in log_{10} copies/mL (No. of specimens). The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL. See Table 2 for expansion of abbreviations.
Figure 3. (A) The mean ± SD viral load (log_{10} copies/mL) profile in NPA at different days post symptom onset in pandemic A(H1N1)-infected patients not treated and treated with oseltamivir. The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL. (B) The percentage of pandemic A(H1N1)-infected patients with detectable virus in respiratory specimens at different days post oseltamivir initiation in those with oseltamivir initiated ≤ 2 and > 2 days post symptom onset. See Figure 1 legend for expansion of abbreviations.

Table 5—Viral Load in NPA at Different Days Post-Symptom Onset in Pandemic A(H1N1)-Infected Patients Not Treated and Treated With Oseltamivir

| Patients | No. of d Post-Symptom Onset |
|----------|-----------------------------|
|          | 0  | 1   | 2   | 3   | 4   | 5   | 6   | 7   |
| Nontreated | 8.59 ± 0.71 (4) | 7.21 ± 1.64 (3) | 6.52 ± 0.20 (2) | 7.39 ± 0.97 (8) | 6.51 ± 2.29 (3) | 7.29 ± 1.55 (3) | 5.03 ± 1.76 (5) | 5.66 ± 1.41 (4) |
| Treated   | 7.83 ± 1.27 (7) | 7.64 ± 1.91 (38) | 6.86 ± 2.03 (15) | 7.21 ± 1.75 (12) | 6.32 ± 2.01 (10) | 5.03 ± 1.50 (11) | 6.06 ± 1.89 (8) | 4.58 ± 2.52 (11) |
| P value   | .304 | .709 | .822 | .799 | .887 | .040 | .349 | .440 |

Values given are mean ± SD viral load in log_{10} copies/mL (no. of specimens). The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL. See Table 2 for expansion of abbreviations.
Despite the apparent efficacy of oseltamivir in mild cases, its efficacy in stopping further disease progression of late cases remains uncertain.

A randomized control treatment trial is not possible at the beginning of the epidemic in our locality because of the uncertainties of its disease severity and the international recommendations on oseltamivir treatment. Moreover, patients presented to us on different days post symptom onset and some have refused further nasopharyngeal sampling once their symptoms improved. Despite these limitations, the nontreated cases are still comparable to the treated patients in demographics, symptoms, and laboratory findings. A previous viral load study of pandemic A(H1N1) infection focused on oseltamivir-treated patients; this is the first study, to our knowledge, of natural viral load profile of these patients without such treatment. Despite the apparent efficacy of oseltamivir in mild cases, its efficacy in stopping further disease progression of late cases remains uncertain.

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**Table 6**—Viral Load of NPA at Different Intervals Post Symptom Onset in Pandemic A(H1N1)-Infected Patients Not Treated and Treated With Oseltamivir ≤ 2 and > 2 d of Symptom Onset

| Oseltamivir Treatment Status | Intervals Post Symptom Onset, d | 0-1 | 2-3 | 4-5 | 6-7 |
|-----------------------------|---------------------------------|-----|-----|-----|-----|
| Nontreated                  |                                 | 8.00 ± 1.30 (7) | 7.22 ± 0.94 (10) | 6.90 ± 1.80 (6) | 5.64 ± 1.27 (8) |
| Treated                     |                                 | 7.63 ± 1.86 (42) | 6.88 ± 1.90 (18) | 5.09 ± 1.41 (13) | 3.93 ± 1.43 (13) |
| Time of initiation, days of symptom onset ≤ 2 | | .621 | .607 | .029 | .012 |
| Time of initiation, days of symptom onset > 2 | | 8.15 ± 1.11 (3) | 7.28 ± 1.93 (9) | 6.54 ± 2.17 (8) | 7.96 ± 1.19 (2) |
| P valuea | | .864 | .920 | .749 | .005 |
| P valuet | | .638 | .609 | .078 | <.001 |

Values given are mean ± SD viral load in log_{10} copies/mL (No. of specimens). The detection limit of the quantitative RT-PCR was 2.95 log_{10} copies/mL.

- Comparison between nontreated and treated patients with oseltamivir initiated ≤ 2 d of symptom onset.
- Comparison between nontreated and treated patients with oseltamivir initiated > 2 d of symptom onset.
- Comparison between treated patients with treatment initiated ≤ 2 and > 2 d of symptom onset.
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Dr Cheng: contributed to collecting the clinical data and samples.
Dr Tsang: contributed to collecting the clinical data and samples.
Dr Lau: contributed to collecting the clinical data and samples.
Dr Lam: contributed to designing, executing, and supervising the study.

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REFERENCES
1. World Health Organization. Human infection with new influenza A (H1N1) virus: clinical observations from Mexico and other affected countries, May 2009. Wkly Epidemiol Rec. 2009;84(21):185-189.
2. World Health Organization. Statement to the press by WHO Director-General Dr Margaret Chan June 11, 2009. World now at the start of 2009 influenza pandemic. http://www.who.int/mediacentre/news/stories/2009/h1n1_pandemic_phase6_20090611/en/index.html. Accessed August 3, 2009.
3. Centers for Disease Control and Prevention (CDC). Update: novel influenza A (H1N1) virus infection-Mexico, March-May, 2009. MMWR Morb Mortal Wkly Rep. 2009;58(21):585-589.
4. Hancock K, Veguilla V, Lu X, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. N Engl J Med. 2009;361(20):1945-1952.
5. Mainey TR, Jayaraman A, Behser JA, et al. Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. Science. 2009;325(5939):484-487.
6. Munster VJ, de Wit E, van den Brand JMA, et al. Pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets. Science. 2009;325(5939):481-483.
7. Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of swine-origin H1N1 influenza virus. Nature. 2009;459(7249):93-93.
8. Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, et al. INER Working Group on Influenza. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N Engl J Med. 2009;361(7):680-689.
9. World Health Organization. WHO Guidelines for pharmacological management of pandemic (H1N1) 2009 influenza and other influenza virus. 20 Aug 2009. http://www.who.int/csr/resources/publications/swineflu/h1n1_guidelines_pharmaceutical_mngst.pdf. Accessed November 30, 2009.
10. Hospital Authority. Hong Kong SAR. HA Central Committee on Infections Disease and Emergency Responses (CCIDER). Interim guidance on antiviral treatment, chemoprophylaxis and pneumococcal vaccination for human swine influenza (H5N1) / influenza A (H1N1) infection. http://www.ha.org.hk/hsflu/hsflu/Interim_Treatment.pdf. Accessed August 5, 2009.
11. Centers for Disease Control and Prevention. CDC Health Alert Network (HAN) Info Service Message: Three reports of oseltamivir-resistant novel influenza A (H1N1) viruses. http://www.cdc.gov/h1n1flu/HAN/070909.htm. Accessed August 3, 2009.
12. Wong SS, Yuen KY. Avian influenza virus infections in humans. Chest. 2006;129(1):156-168.
13. Nicholson KG, Aoki FY, Osterhaus AD, et al; Neuraminidase Inhibitor Flu Treatment Investigator Group. Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Lancet. 2000;355(9218):1845-1850.
14. Wong SS, Yuen KY. Antiviral therapy for respiratory tract infections. Respiriolo. 2008;13(7):956-971.
15. Jefferson T, Jones M, Doshi P, Del Mar C. Neuraminidase inhibitors for preventing and treating influenza in healthy adults: systematic review and meta-analysis. BMJ. 2009;339:b5106.
16. Food and Health Bureau. The Government of the Hong Kong SAR. Legislative Council Panel on Health Services. Prevention and control of human swine influenza infection in Hong Kong. http://www.legco.gov.hk/yr08-09/english/panels/lhs/papers/hs0511chb2-1505-1-e.pdf. Accessed August 3, 2009.
17. Centre for Health Protection, Department of Health, Hong Kong SAR. Strategy and management of human swine influenza (H5N1). http://www.chp.gov.hk/files/pdf/Strategy_swine_flu_20090512_Eng.pdf. Accessed August 3, 2009.
18. Hospital Authority. Hong Kong SAR. HA Central Committee on Infectious Disease and Emergency Responses (CCIDER). Interim guidance on antiviral treatment, chemoprophylaxis and pneumococcal vaccination for human swine influenza (H5N1) / influenza A (H1N1) infection. http://www.ha.org.hk/hsflu/hsflu/Interim_Treatment.pdf. Accessed August 5, 2009.
19. Cheng VC, Hung IF, Tang BS, et al. Viral replication in the nasopharynx is associated with diarrhea in patients with severe acute respiratory syndrome. Clin Infect Dis. 2004;38(4):467-475.
20. Boivin G, Hardy I, Tellier G, Mazziade J. Predicting influenza infections during epidemics with use of a clinical case definition. Clin Infect Dis. 2000;31(5):1166-1169.
21. Zambron M, Hays J, Webster A, Newman R, Keene O. Diagnosis of influenza in the community: relationship of clinical diagnosis to confirmed virological, serological, or molecular detection of influenza. Arch Intern Med. 2001;161(17):2116-2122.
22. Chan KH, Yam WC, Pang CM, et al. Comparison of the Nuclisens easyMAG and Qiagen BioRobot 9604 nucleic acid extraction systems for detection of RNA and DNA respiratory viruses in nasopharyngeal aspirate samples. J Clin Microbiol. 2008;46(7):2195-2199.
23. To KK, Chan KH, Li IW, et al. Viral load in patients infected with pandemic H1N1 2009 influenza A virus. J Med Virol. 2010;82(1):1-7.
24. Cheng VC, Tang BS, Wu AK, Chu CM, Yuen KY. Medical treatment of viral pneumonia including SARS in immunocompetent adult. J Infect. 2004;49(4):262-273.
25. Centers for Disease Control and Prevention (CDC). CDC Realtime RT-PCR (rRTPCR) protocol for detection and characterization of influenza (version 2007). CDC REF. # I-007-05.
26. Li IW, Chan KH, To KW, et al. Differential susceptibility of different cell lines to swine-origin influenza A H1N1, seasonal human influenza A H1N1, and avian influenza A H5N1 viruses. J Clin Virol. 2009;46(4):325-330.
27. Hayden FG, Trenar JJ, Fritz RS, et al. Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza: randomized controlled trials for prevention and treatment. JAMA. 1999;282(13):1240-1246.
28. Sato M, Hosoya M, Kato K, Suzuki H. Viral shedding in children with influenza virus infections treated with neuraminidase inhibitors. Pediatr Infect Dis J. 2005;24(10):931-932.
29. Meijer A, Lackenby A, Hungnes O, et al; European Influenza Surveillance Scheme. Oseltamivir-resistant influenza virus A (H1N1), Europe, 2007-08 season. Emerg Infect Dis. 2009;15(4):552-560.
30. de Jong MD, Tran TT, Truong HK, et al. Oseltamivir resistance during treatment of influenza A (H5N1) infection. N Engl J Med. 2005;353(25):2667-2672.
31. Yuen KY, Chan PK, Peiris M, et al. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. Lancet. 1998;351(9101):467-471.
32. Kiso M, Mitamura K, Sakai-Tagawa Y, et al. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. Lancet. 2004;364(9436):759-765.
33. Hauge SH, Dudman S, Borgen K, Lackenby A, Hungnes O. Oseltamivir-resistant influenza A (H1N1), Norway, 2007-08. Emerg Infect Dis. 2009;15(2):155-162.
34. Centers for Disease Control and Prevention (CDC). Update: drug susceptibility of swine-origin influenza A (H1N1) viruses, April 2009. MMWR Morb Mortal Wkly Rep. 2009;58(16):433-435.
35. Kawai N, Ikematsu H, Hirotsu N, et al. Clinical effectiveness of oseltamivir and zanamivir for treatment of influenza A virus subtype H1N1 with the H274Y mutation: a Japanese, multicenter study of the 2007-2008 and 2008-2009 influenza seasons. Clin Infect Dis. 2009;49(12):1828-1835.
36. Chen H, Cheung CL, Tai H, et al. Oseltamivir-resistant influenza A pandemic (H1N1) 2009 virus, Hong Kong, China. Emerg Infect Dis. 2009;15(12):1970-1972.
37. Jegerlehner A, Schmitz N, Storni T, Bachmann MF. Influenza A vaccine based on the extracellular domain of M2: weak protection mediated via antibody-dependent NK cell activity. J Immunol. 2004;172(9):5598-5605.
38. Cheng VCC, Lau YK, Lee KL, et al. Fatal co-infection with swine origin influenza virus A/H1N1 and community-acquired methicillin-resistant Staphylococcus aureus. J Infect. 2009;59(5):366-370.
39. Wong SS, Yuen KY. The severe acute respiratory syndrome (SARS). J Neurovirol. 2005;11(5):455-468.
40. Hung IF, Cheng VC, Wu AK, et al. Viral loads in clinical specimens and SARS manifestations. Emerg Infect Dis. 2004;10(9):1550-1557.
41. Zhou J, Law HK, Cheung CY, Ng IH, Peiris JS, Lau YL. Functional tumor necrosis factor-related apoptosis-inducing ligand production by avian influenza virus-infected macrophages. J Infect Dis. 2006;193(7):945-953.
42. Miller RR III, Markewitz BA, Rolfs RT et al. Clinical findings and demographic factors associated with intensive care unit admission in Utah due to novel 2009 influenza A(H1N1) infection. Chest. 2010;137(4):752-758.
43. Centers for Disease Control and Prevention (CDC). Hospitalized patients with novel influenza A (H1N1) virus infection - California, April - May, 2009. MMWR Morb Mortal Wkly Rep. 2009;58(19):536-541.
44. Goodlons J, Jonges M, Claas EC, Meijer A, Kroes AC. Prolonged influenza virus infection during lymphocytopenia and frequent detection of drug-resistant viruses. J Infect Dis. 2009;199(10):1435-1441.
45. Daley P, Castriciano S, Chernesky M, Smejka M. Comparison of flocked and rayon swabs for collection of respiratory epithelial cells from uninfected volunteers and symptomatic patients. J Clin Microbiol. 2006;44(6):2265-2267.
46. Kitching A, Roche A, Balasegaram S, Heathcock B, Maguire H. Oseltamivir adherence and side effects among children in three London schools affected by influenza A(H1N1)v, May 2009 - an internet-based cross-sectional survey. Euro Surveill. 2009;14(30):19287. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19287.
47. Toovey S, Rayner C, Prinssen E, et al. Assessment of neuropsychiatric adverse events in influenza patients treated with oseltamivir: a comprehensive review. Drug Saf. 2008;31(12):1097-1114.
48. Huang YC, Li WC, Tsao KC, Huang CG, Chiu CH, Lin TY. Influenza associated central nervous system dysfunction in Taiwanese children: clinical characteristics and outcomes with and without administration of oseltamivir. Pediatr Infect Dis J. 2009;28(7):647-648.