rigorous, protocol-driven ventilation with low tidal volumes (an approach demonstrated to improve mortality in the Acute Respiratory Management in ARDS trial) is associated with impressive survival rates, even in patients with profoundly hypoxemic ARDS.

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A Real-Time Reverse Transcriptase-Polymer Chain Reaction To Evaluate Natural History of Viral Shedding in Outpatient Children and Adolescents With Pandemic 2009 Influenza A(H1N1)

To the Editor:

Li et al report in a recent issue of CHEST (April 2010) a retrospective cohort study in which the viral load (VL) profile was evaluated by reverse transcription-polymerase chain reaction (RT-PCR) in 27 untreated patients with pandemic 2009 influenza A(H1N1) [A(H1N1)] and 118 patients treated with oseltamivir. The mean age for the cohort was 17.6 years, but none of the patients was aged <1 year. This study provided interesting data on the effect of oseltamivir on viral shedding in children and adults, but the data are less reliable when describing natural history of shedding in untreated patients. As a matter of fact, VL on nasopharyngeal aspirates (NPAs) were reported in seven patients at 1 day, 10 at 2 to 3 days, six at 4 to 5 days, and eight at 6 to 7 days, thus suggesting that different patients were evaluated at each interval post-symptom onset or that a complete follow-up was performed on six patients at most.

By contrast, we prospectively evaluated VL in NPAs from 23 untreated infants, children, and adolescents (median age, 12.7 years; range, 0.7 to 17 years) without underlying comorbidities and with uncomplicated influenza attending to the Anna Meyer Children’s University Hospital (Florence, Italy) during July 2009. With parental consent, clinical assessment and NPAs were repeated at 5 and 10 days from symptom onset in children with positive results. RNA was extracted by the QIAamp Viral RNA miniKit (Qiagen; Milan, Italy). Two real-time RT-PCRs, using the SuperScript III Platinum One-Step qRT-PCR (Invitrogen; Carlsbad, CA), and primers and probes targeting the influenza A virus M gene and the hemagglutinin gene of the A(H1N1), respectively, were performed. The positive control was the strain A/Italy/05/2009(H1N1) isolated in our laboratory (accession numbers GQ251032 to GQ251039). For the quantitative assay targeting the hemagglutinin gene, serial dilutions (-2 to -7 containing
Table 1—Viral RNA Concentrations in All Positive Nasopharyngeal Aspirates

<table>
<thead>
<tr>
<th>Variable</th>
<th>First NPA</th>
<th>Second NPA</th>
<th>Third NPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>After symptom onset, d (IQR)</td>
<td>1 (0-2)</td>
<td>5.5 (5-6.25)</td>
<td>8 (8-9)</td>
</tr>
<tr>
<td>Children with positive test results, No. (%)</td>
<td>23 (100)</td>
<td>17 (73.9)</td>
<td>4 (17.4)</td>
</tr>
<tr>
<td>Viral RNA concentrations (log_{10} TCID_{50}), mean ± SD</td>
<td>3.86 ± 1.18</td>
<td>3.41 ± 0.88</td>
<td>2.81 ± 0.97</td>
</tr>
</tbody>
</table>

First vs second NPA, \( P = .184 \); second vs third NPA, \( P = .102 \) (comparisons by Student t test). IQR = interquartile range; NPA = nasopharyngeal aspirate; TCID_{50} = tissue culture infectious doses 50 equivalents.

\( N = 23 \).

1 \times 10^{10} \text{ to } 1 \times 10^{9} \) tissue culture infectious doses 50 equivalents (TCID_{50}/mL) of the positive control strain were used to construct the calibration curve. Approximately one TCID_{50} contained 1,000 viral genome copies. The estimated limit of the quantitative RT-PCR was 3.1 TCID_{50} equivalents/mL (approximately 3,100 copies/mL). Virus concentrations (expressed in TCID_{50} equivalents) were log transformed and reported (Table 1) as mean ± SD. One girl still had a positive result 12 days after onset of fever. Duration of fever in all 23 children was 3 days (interquartile range, 3 to 5 days).

Our findings indicate that about three-fourths of children and adolescents longitudinally evaluated and with a mild A(H1N1) illness shed the virus at 5 days and that a small but discrete (17%) proportion shed the virus up to 9 days after symptom onset. VL was evaluated by molecular methods only, which also may amplify viral RNA from nonviable virus particles. However, quantitative VLs detected 9 days after onset of symptoms were compatible with the presence of viable virus, as found in our in vitro study (data not shown). So far, even if the relationship between viral shedding measured by a quantitative RT-PCR and viral transmission has not been clearly demonstrated, observations of prolonged A(H1N1) detection in respiratory specimens from infants, children, and adolescents several days after fever disappearance may have important implications for infection control in the community and in hospital settings.

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**References**


**Response**

To the Editor:

Oseltamivir has been shown to be efficacious in the treatment of pandemic 2009 influenza A(H1N1) virus-[A(H1N1)]-infected patients with mild disease; a significantly lower viral load at day 5 after symptom onset was achieved as compared with nontreated patients. Moreover, a greater rate of viral load reduction in the nasopharyngeal aspirate (NPA) of patients treated with oseltamivir was observed when initiated <2 days after symptom onset. However, the effectiveness and safety of oseltamivir in treating A(H1N1)-infected children aged <1 year was uncertain; therefore, these patients were excluded from the treated and nontreated groups in our study despite the authorized use of oseltamivir during the pandemic in Europe and a number of other countries. Several studies are being performed to address these issues. Nevertheless, the age range of nontreated patients in our study was 5 to 49 years; at least one-fourth (7/27) and 77.8% (21/27) were aged ≤12 and ≤18 years, respectively (unpublished data).

The respiratory specimens of our study were collected at the beginning of the pandemic when a randomized controlled treatment trial was not feasible because of the uncertainties of disease severity and international recommendations on oseltamivir treatment. The viral load of NPA from nontreated patients could only be compared with treated patients with reference to the interval postsymptom onset. The timing of when the first NPA of each patient could be obtained largely depended on their number of days postsymptom onset at presentation, so different numbers of specimens were included at different intervals of postsymptom onset for analysis even when serial samplings were obtained from patients. Some refused further nasopharyngeal sampling once their symptoms improved.

Thirty-seven percent and 9% of A(H1N1)-infected, oseltamivir-treated patients had virus detected by real-time polymerase chain reaction (PCR) in nasal-throat swabs on days 7 and 10 of illness. Similarly, virus was detected in NPA from nontreated and treated patients at days 8 to 9 postsymptom onset (mean viral load, 3.42 ± 1.07 vs 3.51 ± 1.19 log_{10} copies/mL, \( P = .937 \)) (unpublished data). Young children shed seasonal influenza virus for longer duration than adults. Similarly, 50% and 38% of nasopharyngeal swabs from children aged 0 to 9 years had A(H1N1) detected by A(H1N1) PCR at days 8 and 11 postsymptom onset, respectively, whereas only 42% and 20% from adults aged ≥18 had virus detected correspondingly. None of the PCR-positive specimens collected at day 11 postsymptom onset from patients of all age groups had positive virus culture.

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