

# Morbidity and Mortality Associated With Nosocomial Transmission of Oseltamivir-Resistant Influenza A(H1N1) Virus

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**A** GLOBAL EMERGENCE AND rapid spread of oseltamivir-resistant influenza A(H1N1) viruses carrying a neuraminidase (NA) gene with an H274Y (N2 numbering; H275Y in N1 numbering) amino acid substitution has been observed since January 2008.<sup>1-3</sup> Viruses carrying this mutation are presumed to exhibit attenuated pathogenicity,<sup>4</sup> compromised transmission,<sup>5</sup> and reduced lethality.<sup>6</sup> However, current widespread circulation of oseltamivir-resistant influenza A(H1N1) viruses associated with typical influenza illnesses and viral pneumonia suggest that these viruses retain significant transmissibility and pathogenicity.<sup>2,3,7,8</sup> While these resistant variants may cause significant mortality and retain efficient transmission, these properties have not yet been firmly established.

## METHODS

In February 2008, an outbreak of influenza A(H1N1) virus occurred in a medical ward at a Dutch university hos-

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**Context** The sudden emergence and rapid spread of oseltamivir-resistant influenza A(H1N1) viruses with neuraminidase (NA) gene H274Y amino acid substitution is the hallmark of global seasonal influenza since January 2008. Viruses carrying this mutation are widely presumed to exhibit attenuated pathogenicity, compromised transmission, and reduced lethality.

**Objective** To investigate nosocomial viral transmission in a cluster of patients with influenza A(H1N1) virus infection.

**Design, Setting, and Patients** Descriptive outbreak investigation of 2 hematopoietic stem cell transplant recipients and an elderly patient who developed hospital-acquired influenza A virus infection following exposure to an index patient with community-acquired H274Y-mutated influenza A(H1N1) virus infection in a medical ward at a Dutch university hospital in February 2008. The investigation included a review of the medical records, influenza virus polymerase chain reaction and culture, phenotypic oseltamivir and zanamivir susceptibility determination, and hemagglutinin chain 1 (HA<sub>1</sub>) gene and NA gene sequence analysis.

**Main Outcome Measure** Phylogenetic relationship of patient cluster influenza A(H1N1) viruses and other 2007-2008 seasonal influenza A(H1N1) viruses.

**Results** Viral HA<sub>1</sub> and NA gene sequence analysis from the 4 patients revealed indistinguishable nucleotide sequences and phylogenetic clustering of H274Y-mutated, oseltamivir-resistant influenza A(H1N1) virus, confirming nosocomial transmission. Influenza virus pneumonia (3 patients) and attributable mortality (2 patients) during active infection was observed in patients with lymphocytopenia at onset.

**Conclusion** Seasonal oseltamivir-resistant influenza A(H1N1) viruses with NA gene H274Y mutation are transmitted and retain significant pathogenicity and lethality in high-risk patients.

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pital. Clinical specimens from symptomatic contact patients of the presumed index patient were tested by influenza polymerase chain reaction (PCR) and sequences were further analyzed. Medical records of contact patients with related influenza virus infection were reviewed for underlying disease, clinical findings, and outcome. Screening specimens were ob-

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tained from contacts of the last outbreak patient (patient 4) to rule out further spread of the virus.

### Influenza Virus Diagnostics

Influenza virus detection was performed on clinical respiratory specimens using rapid antigen testing and PCR (as described previously<sup>9</sup>), along with viral culture. Antigenic characterization (hemagglutination inhibition testing) and phenotypic oseltamivir and zanamivir susceptibility (IC<sub>50</sub>, concentration of drug needed to inhibit enzyme activity by 50%) were determined as described.<sup>10,11</sup> Viral RNA extracted from clinical specimens was further transcribed and amplified. Hemagglutinin chain 1 (HA<sub>1</sub>) and neuraminidase gene sequences from patients with confirmed influenza virus infection were

analyzed and phylogenetically related to other 2007-2008 seasonal influenza viruses obtained at the hospital or sentinel surveillance isolates collected nationwide using Bionumerics version 5.1 (Applied Maths, Sint-Martens-Latem, Belgium).

### Ethical Considerations

The investigation of this outbreak did not involve any planned activity that could have been reviewed prospectively by an institutional review board or ethics committee. Nevertheless, all necessary precautions were taken to prevent identification of the patients and health care workers involved. The physicians involved all gave signed permission to use clinical data and were informed on the outcome of the investigation. All of them agreed with the in-

tention to publication. Details including the age and role of the health care workers were omitted or described in a nonspecific way, while we also took care to preserve all clinically meaningful details. The chairman of the ethics committee at the Leiden University Medical Center was consulted retrospectively and agreed to the approach as described for reporting the clinical information obtained during the investigation and included herein.

## RESULTS

### Nosocomial Influenza A(H1N1) Virus Outbreak

The clinical characteristics and timeline of the outbreak of influenza A(H1N1) virus are shown in the TABLE and in FIGURE 1, respectively.

**Table.** Clinical Characteristics of 4 Hospitalized Patients With Oseltamivir-Resistant Influenza A(H1N1) Virus Infection

	Patient 1 (Index)	Patient 2	Patient 3	Patient 4
Sex	Male	Female	Male	Male
Age, y	47	57	89	66
Medical history	Systemic lupus erythematosus, sarcoidosis, diabetes	Multiple myeloma, allogeneic stem cell transplantation following reduced-intensity conditioning engraftment	Diabetes, cardiac ischemia	Acute myelogenous leukemia, allogeneic stem cell transplantation following myeloablative conditioning, chronic graft-vs-host disease
Immunosuppressive therapy	Prednisolone	None	None	Mycophenolate mofetil, prednisone, recent cyclosporine
Reason for admission	Fever, dyspnea	Fever, mild cough	Fever	Fever
Confirmed diagnosis	Influenza lower respiratory tract infection	Bacteremia, source unknown	Bacteremia, urosepsis	Bacteremia, abdominal sepsis, active graft-vs-host disease
First positive test result for influenza A <sup>a</sup>	January 29, 2008	February 11, 2008	February 15, 2008	February 14, 2008
No. of days following admission in hospital	0	16	17	17
Antiviral therapy	Oseltamivir	Oseltamivir	Oseltamivir	Oseltamivir
Absolute lymphocyte count within 48 h of symptom onset, cells/ $\mu$ L	699 (lymphocytopenia)	3195 (normal count)	726 (lymphocytopenia)	217 (lymphocytopenia)
Respiratory symptoms	Dyspnea, oxygen dependent	Cough	Dyspnea, oxygen dependent	Dyspnea, oxygen dependent
Chest radiograph result	Left lower-lobe consolidation	No pulmonary abnormalities	Bilateral lower-lobe consolidations	Perihilar consolidations
Absolute lymphocyte count during follow-up, cells/ $\mu$ L <sup>b</sup>	1134 (normal count)	4739 (normal count)	Not available	129 (lymphocytopenia)
Influenza outcome	Viral clearance	Viral clearance	Fatal influenza pneumonia <sup>c</sup>	Fatal influenza pneumonia <sup>c,d</sup>

<sup>a</sup>Determined by polymerase chain reaction (PCR).

<sup>b</sup>Lymphocyte count determined 2 to 6 weeks after influenza confirmation.

<sup>c</sup>Clinical diagnosis (dyspnea, PCR detection of influenza A in respiratory specimens).

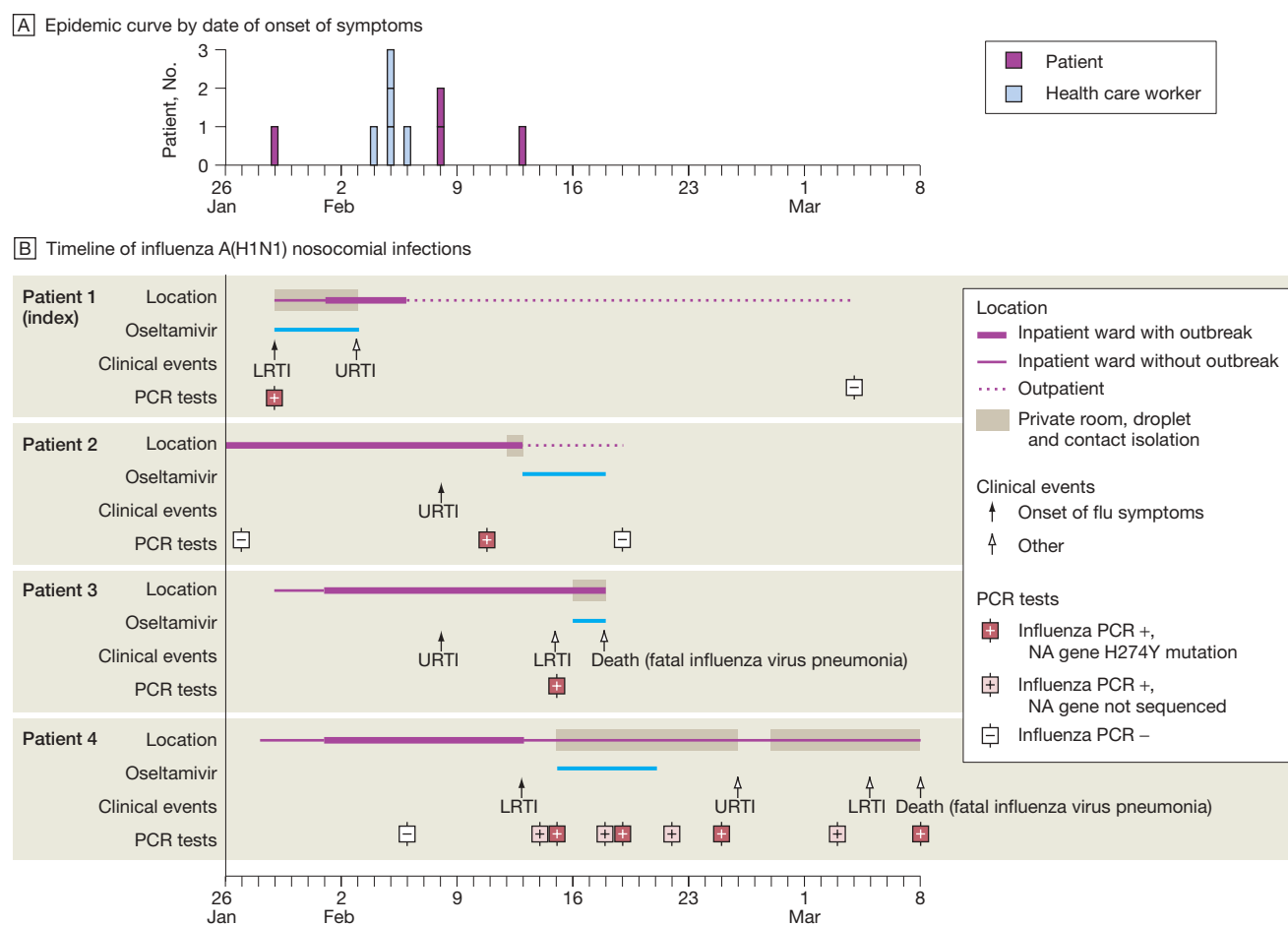
<sup>d</sup>Pathological diagnosis (histological features, PCR detection of influenza A in pulmonary tissue).

Community-acquired oseltamivir-resistant influenza A(H1N1) virus with NA gene H274Y mutation, isolated from the presumed index case, was detected in 3 additional patients (mean oseltamivir  $IC_{50}$ , 484 nM; mean zanamivir  $IC_{50}$ , 1.1 nM). The presumed index case (patient 1), who was vaccinated for 2007-2008 seasonal influenza and received high-dose (cumulative) prednisolone therapy for systemic lupus erythematosus, was admitted to the hospital on January 29, 2008, with fever, cough, dyspnea, and lymphocytopenia. Mechanical ventilation and broad-spectrum empirical antibacterial treatment were initiated for acute

respiratory failure and apparent pulmonary consolidations by chest radiography. Oseltamivir administration was initiated following influenza A virus detection using rapid antigen testing and PCR along with contact and droplet isolation. No other viral and bacterial respiratory pathogens were detected and blood cultures remained negative. The patient was transferred to a medical ward following clinical improvement on February 1 and isolation precautions were continued for the duration of symptoms until February 3. Viral clearance was confirmed by PCR upon lymphocyte reconstitution in an outpatient setting on March 4, 2008.

Two hematopoietic stem cell transplant recipients (patient 2 and patient 4) and an 89-year-old elderly patient (patient 3) developed hospital-acquired influenza A virus infection (Table). These patients were present at the same time as the index patient at the medical ward without isolation procedures (Figure 1), but the 4 patients never shared rooms. Patient 2 developed mild influenza symptoms with rapid viral clearance, whereas both patients 3 and 4 developed pulmonary consolidations and fatal respiratory failure with viral excretion under broad-spectrum antibacterial therapy. The influenza vaccination status of these patients is not known. The attribution

**Figure 1.** Epidemic Curve by Date of Onset of Symptoms and Timeline of Influenza A(H1N1) Nosocomial Infections



During influenza A(H1N1) virus outbreak, all 4 infected patients were admitted in the same department and never shared rooms. LRTI indicates lower respiratory tract infection; NA, neuraminidase; PCR, polymerase chain reaction; and URTI, upper respiratory tract infection.

of mortality to influenza was supported by detection of influenza A(H1N1) viral RNA from postmortem pulmonary tissue and histopathological pulmonary findings consistent with viral pneumonia in patient 4, with the exclusion of other pathogens. Further

nosocomial spread to other contacts within the wards was excluded by PCR.

### Phylogenetic Relationship

HA<sub>1</sub> gene and NA gene sequence analysis of viruses from the 4 outbreak patients revealed indistinguishable

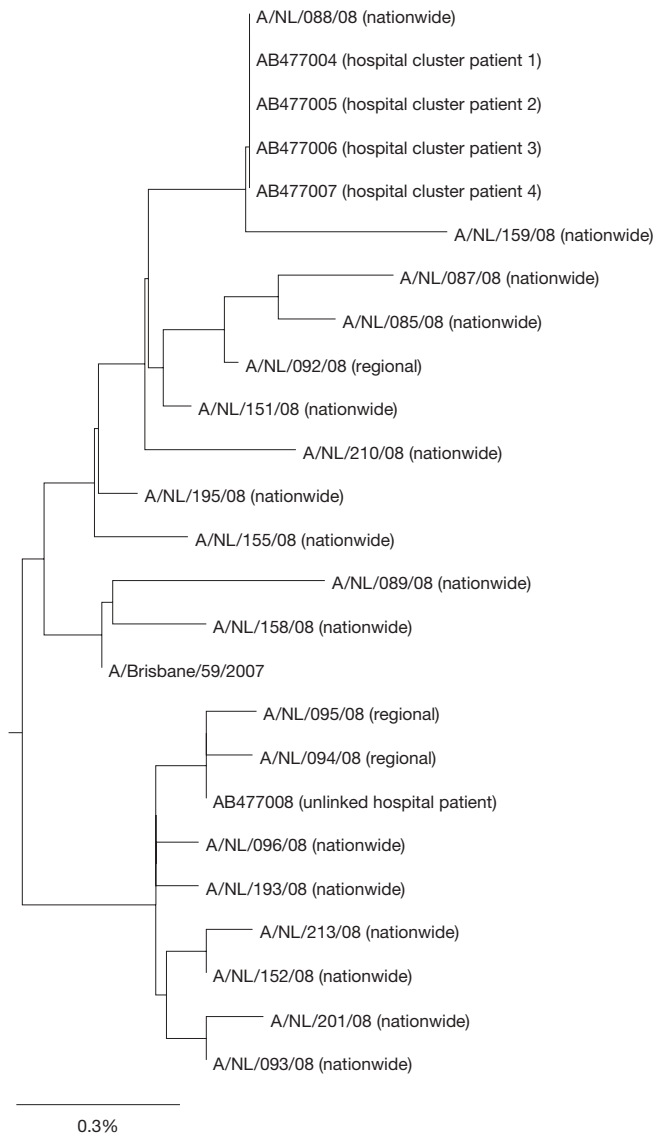
nucleotide sequences and phylogenetic clustering (FIGURE 2). In addition to NA gene H274Y substitution, a rare T284A substitution was sequenced (NA gene sequence GenBank accession numbers AB476754, AB476755, AB476756, AB462370) and could therefore be recognized as a marker for transmission. An unlinked national surveillance isolate (A/NL/088/08) with identical HA<sub>1</sub> gene nucleotide sequence revealed no NA gene phylogenetic clustering and lacked the specific T284A substitution. The NA gene T284A mutation was not observed in other 2007-2008 seasonal influenza A(H1N1) viruses or in worldwide sequences submitted to public databases, reinforcing the unique genetic relatedness of this influenza A(H1N1) virus patient cluster.

### Health Care Workers

Five health care workers developed influenza-like illness (onset February 4-6, 2008) during admission of the presumed index patient. One health care worker, vaccinated for the 2007-2008 seasonal influenza, developed influenza-like illness following established contact with the index patient and continued working. Four other health care workers took sick leave within 24 to 48 hours of symptom onset. However, no samples for influenza testing were obtained from any of these 5 health care workers. Thus, their role in possibly contributing to this apparent nosocomial spread of influenza could not be confirmed.

Viruses cultured from the patient cluster revealed a poor antigenic match with the 2007-2008 vaccine reference strain A/Solomon Islands/3/06 (approximately 16-fold difference by duplicate hemagglutination inhibition testing). This may in part explain the sustained susceptibility and infectivity of the vaccinated index patient and suspected health care worker resulting in the hospital outbreak.

**Figure 2.** Phylogenetic Relationship of Nosocomial Patient Cluster Influenza A(H1N1) Viruses and Other 2007-2008 Seasonal Influenza A Viruses



Influenza A(H1N1) virus HA<sub>1</sub> gene sequences obtained from the patient cluster (n=4, marked as hospitalized cluster patient 1, 2, 3, 4) were related to available unlinked 2007-2008 seasonal influenza A(H1N1) viral sequences obtained at the hospital (n=1, marked as unlinked hospitalized patient), surveillance isolates collected within a 10-km regional zone from the hospital (n=3, marked as regional), nationwide collected surveillance isolates (n=17, marked as nationwide), and vaccine strain A/Brisbane/59/2007. The HA<sub>1</sub> gene (nucleotides 1-1071) neighbor-joining tree was rooted on vaccine strain A/Solomon Islands/3/06. Viral sequence GenBank accession numbers are depicted for hospitalized patients.

### COMMENT

This outbreak provided evidence that circulating oseltamivir-resistant influenza A(H1N1) viruses with NA gene H274Y mutation are transmitted be-

tween humans. Limitations of this observational study include the small number of patients, therefore the findings require careful interpretation and do not allow conclusions on the frequency of this complication in hospital settings. The vaccination status of secondarily infected cases (patients 2, 3, and 4) remained unclarified. Information on the mechanism of spread was limited by the circumstances in this study. Data obtained from clinical specimens suggest different routes of transmission; however, this could not be further explored because the sampling of symptomatic health care workers and testing of fomites are not routinely performed. However, analysis of data obtained from clinical specimens provided some insight to different routes of transmission and suggested a limited viral spread.

Early identification and prolonged isolation precautions appear prudent in the care for infected immunocompromised patients to prevent nosocomial influenza virus outbreaks. This study confirmed that circulating H274Y-mutated A(H1N1) viruses can retain significant pathogenicity and lethality, as shown in these elderly or immunocompromised patients with lymphocytopenia, underlining the urgency for the introduction of new effective antiviral agents and therapeutic strategies.<sup>12</sup>

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**Author Contributions:** Dr Gooskens had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Gooskens, Claas, van den Broek, Kroes.

**Acquisition of data:** Gooskens, Jonges, Claas.

**Analysis and interpretation of data:** Gooskens, Jonges, Claas, Meijer, Kroes.

**Drafting of the manuscript:** Gooskens, van den Broek.

**Critical revision of the manuscript for important intellectual content:** Gooskens, Jonges, Claas, Meijer, Kroes.

**Statistical analysis:** Jonges.

**Obtained funding:** Meijer.

**Administrative, technical, or material support:** Jonges, Claas.

**Study supervision:** Gooskens, Claas, Meijer, van den Broek, Kroes.

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