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## Pandemic (H1N1) 2009

# Oseltamivir-resistant pandemic (H1N1) 2009 influenza in a severely ill patient: the first Australian case

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*After a 10-day course of oral oseltamivir for pandemic (H1N1) 2009 influenza infection, a renal transplant recipient developed rapid-onset severe primary viral pneumonia due to oseltamivir-resistant virus. Respiratory failure progressed despite high-dose oral oseltamivir, nebulised zanamivir and cessation of immunosuppressive medications, but his condition improved with intravenous zanamivir. He subsequently died of non-respiratory complications. This is the first case of oseltamivir-resistant pandemic (H1N1) 2009 in Australia and the first report of resistance in a solid organ transplant recipient.*

## Clinical record

A 38-year-old cadaveric renal transplant recipient had remained well on a standard immunosuppressive regimen until he presented 7 weeks after transplantation with coryzal symptoms and fever. Nose and throat swabs were collected for influenza testing, and he was empirically commenced on oral oseltamivir at the recommended dose of 75 mg twice a day,<sup>1,2</sup> to be taken at home. The swabs were subsequently confirmed as positive for pandemic (H1N1) 2009 influenza ([Box](#)), and he continued taking oseltamivir for 10 days, with clinical improvement.

Three days after ceasing treatment, the patient was admitted with high fever, myalgia and a cough productive of clear sputum. A chest x-ray showed pulmonary infiltrates in both upper–mid zones and the right lower zone. He was commenced on antibiotics for presumptive community-acquired pneumonia, but over the next 5 days he became hypoxic and tachypnoeic with increasing lung infiltrates and required intubation for ventilatory support. His nose and throat swabs taken on admission were negative for influenza, but 2 days after admission, a sputum sample was positive for pandemic (H1N1) 2009, as was a bronchoalveolar lavage performed 7 days after admission. His

antimicrobial therapy was broadened and oral oseltamivir reintroduced at 75 mg twice daily, despite renal dysfunction, as higher oral doses have been shown to achieve therapeutic blood levels in severely ill patients.<sup>3</sup>

On Day 11, oseltamivir was reduced to 75 mg daily due to anuria, but the patient's absorption of oral fluids subsequently ceased and pandemic (H1N1) 2009 was again detected by polymerase chain reaction (PCR) testing of samples taken on Days 17 and 19. Nebulised zanamivir 15 mg four times a day was added on Day 23, and from this point on, influenza was not detected from respiratory specimens. His immunosuppressive therapy was reduced and subsequently ceased on Day 25, but his condition continued to deteriorate, with worsening lung infiltrates and a partial pressure of oxygen/fraction of inspired oxygen ( $\text{PaO}_2/\text{FiO}_2$ ) ratio of 55 ( $\text{PaO}_2/\text{FiO}_2$  ratio < 300 indicates acute lung injury) by Day 29.

On Day 30, we identified an oseltamivir resistance mutation in the neuraminidase (NA) gene — a point mutation resulting in a histidine-to-tyrosine substitution at position 275 (H275Y) — in the virus from the patient's Day 3 sample, though it was not present in virus from his first illness preceding admission. As his clinical progress with nebulised zanamivir was still poor, intravenous zanamivir was obtained through an emergency investigational drug application for compassionate use and commenced at a modified dose of 60 mg twice a day, based on a predicted ultrafiltration rate of 10 mL/min. Two days later, this was increased to 150 mg twice a day; however, due to a sudden deterioration in his liver function tests (alanine aminotransferase, 825 U/L [normal, < 40 U/L]), the dose was reduced 3 days later to 60 mg twice daily, with prompt improvement in liver function. Oral oseltamivir and nebulised zanamivir were also continued because of uncertainty about the correct intravenous zanamivir dose for a patient on continuous venovenous haemofiltration.

After 7 days of treatment with intravenous zanamivir, the patient's condition slowly improved and he eventually became ventilator-independent, but he subsequently died on Day 78 from intraperitoneal sepsis. At that time he had ceased all antiviral therapy, and repeated tests of upper and lower respiratory tract samples were negative for influenza. Surveillance of close contacts for respiratory illness found no evidence of acquisition of pandemic (H1N1) 2009. During his admission, he was isolated in a single room under full respiratory precautions.

For virological testing of respiratory specimens, dry swabs were tested by PCR only, while swabs in viral transport medium and other fluid specimens were also cultured for influenza virus using a centrifuge-enhanced shell vial culture in Madin–Darby canine kidney cells. Real-time reverse transcription PCR (rRT-PCR) assays were directed at the matrix genes of influenza A and B and the haemagglutinin genes of seasonal A/H1, A/H3 and pandemic (H1N1) 2009 viruses. Cycle threshold (CT) values  $\leq 40$  were regarded as positive. Samples and cultures testing positive for pandemic (H1N1) 2009 were then tested for oseltamivir resistance (Box) using an NA gene rRT-PCR with separate probes specific for the wild-type sequence and the H275Y mutation, by sequencing of the NA gene product (ABI Prism 3130x/ Genetic Analyzer, Applied Biosystems Inc, Foster City, Calif, USA) for the presence of the H275Y mutation, and by testing phenotypic susceptibility to oseltamivir on isolated viruses using a fluorometric assay to determine the oseltamivir 50% inhibitory concentration ( $\text{IC}_{50}$ ).<sup>4</sup>

Samples collected during the patient's first illness contained only wild-type pandemic

(H1N1) 2009, and the isolate collected prior to antiviral therapy was phenotypically susceptible to oseltamivir, with a low  $IC_{50}$  of 0.43 nM (Box). In contrast, all samples testing positive to influenza during his admission contained the mutant strain, with a markedly elevated  $IC_{50}$  of 382.5 nM for the Day 7 isolate, compared with 649.9 nM for the reference oseltamivir-resistant pandemic (H1N1) 2009 virus strain A/Osaka/180/2009. Interestingly, the Day 7 sputum sample appeared to contain a mixture of wild-type and mutant virus on the rRT-PCR test, and sequencing of the Day 19 sample also indicated a possible mixed infection.

## Discussion

To our knowledge, this is the first report of infection with oseltamivir-resistant pandemic (H1N1) 2009 virus in Australia, and the first such report in a solid organ transplant recipient with an ultimately fatal outcome.

Oseltamivir and zanamivir act by specifically inhibiting the NA of influenza viruses. The H275Y mutation — the major mechanism for oseltamivir resistance in influenza A/H1N1 — was rare until it emerged in seasonal influenza A/H1N1 in 2007, but it soon became the dominant type throughout the world,<sup>5</sup> including Australia.<sup>6</sup> Importantly, these oseltamivir-resistant viruses remain susceptible to zanamivir.

As of 9 October 2009, there had been only 31 reports of oseltamivir resistance in pandemic (H1N1) 2009 influenza virus,<sup>7</sup> despite the large amounts of oseltamivir used internationally. All of these resistant viruses have contained the H275Y NA mutation. Most cases have occurred in patients taking oseltamivir as post-exposure prophylaxis, with few in immunosuppressed patients on long-term oseltamivir treatment.<sup>8</sup> One immunosuppressed patient was treated with intravenous zanamivir after inhaled zanamivir was not tolerated.<sup>9</sup> All reported cases due to resistant virus have been sporadic, with no evidence for onward transmission, and no fatal cases have been reported previously.

Our patient was initially infected with wild-type virus, but his first positive sample following relapse, collected 5 days after ceasing his initial course of oseltamivir and 3 days before its reintroduction, detected only oseltamivir-resistant virus. This suggests that the resistant virus emerged in the few days after ceasing his first course of oseltamivir, possibly due to declining blood and tissue levels of the antiviral drug.

During his subsequent admission, the oseltamivir-resistant strain predominated, but there was evidence of a persistent mixed infection, based on the detection of both wild-type and resistant virus by rRT-PCR on Day 7 of admission, and supported by the intermediate elevation of  $IC_{50}$  of that isolate. Interestingly, despite the emergence of the resistant virus, there were declining levels of both resistant and susceptible virus, in spite of the patient's clinical deterioration and even before commencing zanamivir on Day 23, as indicated by a progressive increase in the rRT-PCR CT values (Box). In accordance with our policy for patients with proven or suspected lower respiratory tract involvement, we used twice the standard dose of oseltamivir, adjusted for the patient's renal function. It is possible the oseltamivir therapy had continued to provide some useful antiviral activity, but we were unable to test blood levels of oseltamivir carboxylate to investigate this further.

Despite the negative virological results from respiratory specimens taken while he was

receiving nebulised zanamivir and oral oseltamivir, our patient's condition continued to deteriorate, with dense pulmonary consolidation. There was concern that he was not getting adequate levels of antiviral drug in the lung tissue, so intravenous zanamivir was introduced. As there was no suitable established treatment regimen for patients on continuous venovenous haemofiltration, dosing was based on the ultrafiltration rate. This was calculated as 10 mL/min, correlating to an intravenous zanamivir dose of 60 mg twice daily, but was subsequently increased to 150 mg twice daily due to the patient's continued respiratory deterioration. Liver function rapidly deteriorated following the dose increase but promptly recovered when the dose was reduced, suggesting that the deterioration had been drug-related hepatic dysfunction.

After 7 days of intravenous zanamivir, the patient's respiratory function had stabilised. This may have been an effect of the intravenous zanamivir and/or his improving immune function following the earlier cessation of immunosuppressive therapy. After cessation of his antiviral therapy, there was no evidence of reappearance of the virus, and his death 38 days later was due to non-respiratory complications.

The management of this patient was complicated by the uncertainty surrounding correct dosing of oseltamivir and zanamivir in a severely unwell patient, with the inability to perform therapeutic drug monitoring, and the difficulties in interpreting non-invasive respiratory specimen virological results in the setting of dense pulmonary consolidation. Where absorption is suspected to be unreliable, intravenous zanamivir may prove to be a useful antiviral therapy for severely unwell influenza patients, including those with oseltamivir-resistant pandemic (H1N1) 2009 infection.

Clinicians caring for immunosuppressed patients with pandemic (H1N1) 2009 should be aware of the potential for development of oseltamivir resistance during therapy and for prolonged viral shedding. Strict adherence to infection control measures is recommended until immunosuppressed patients have clinically improved and respiratory specimens test negative by both PCR and viral culture.

#### Laboratory testing results for detection of influenza

Day of admission	Sample	Cycle threshold value (> 40 considered negative)						Oseltamivir IC <sub>50</sub> of cultured virus
		Influenza A matrix gene	Pandemic (H1N1) 2009 H gene	NA gene wild-type	NA gene H275Y mutant	NA gene sequence		
- 13	Nose/throat swabs	29	29	32	Negative	Wild-type*	0.43 nM	
- 9	Nose/throat swabs	Negative	40	Negative	Negative	Wild-type*	—	
1	Nose/throat swabs	Negative	Negative	—	—	—	—	
3	Sputum	17	22	Negative	28	H275Y mutant*	—	

7	Sputum	17	22	35	27	H275Y mutant†	382.5 nM
7	Bronchoalveolar lavage	19	23	Negative	24	H275Y mutant‡	—
8	Nose/throat swabs	23	25	Negative	27	H275Y mutant‡	—
17	Nose/throat swabs	32	40	Negative	38	H275Y mutant*	—
19	Sputum	35	34	37	Negative	Mixed*	—
24	Nose/throat swabs	Negative	Negative	—	—	—	—
24	Sputum	Negative	Negative	—	—	—	—
28	Endotracheal aspirate	Negative	Negative	—	—	—	—
28	Nose/throat swabs	Negative	Negative	—	—	—	—
33	Nose/throat swabs	Negative	Negative	—	—	—	—
33	Endotracheal aspirate	Negative	Negative	—	—	—	—

NA = neuraminidase. IC<sub>50</sub> = 50% inhibitory concentration. — = test not done. \* Sequence from patient sample only. † Sequence from cell culture isolate only. Wild-type virus was detected in this sample by polymerase chain reaction, but sequencing detected only the mutant strain. ‡ Sequence from patient sample and cell culture isolate.

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## Competing interests

David Smith sits on two advisory committees that receive sponsorship from Roche Pharmaceuticals.

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