Preferential Lower Respiratory Tract Infection in Swine-Origin 2009 A(H1N1) Influenza

Ellen Yeh,[1] Robert F. Luo,[1] LauraLe Dyner,[2] David K. Hong,[2] Niaz Banaei,[1,3] Ellen Jo Baron,[1,3] and Benjamin A. Pinsky[1]

Departments of [1]Pathology and [2]Pediatrics, Stanford University School of Medicine, Stanford, and [3]Clinical Virology Laboratory, Stanford University Hospital and Lucile Packard Children's Hospital, Palo Alto, California

We report a case of 2009 influenza A(H1N1) virus infection in which virus was detected predominantly in specimens from the lower respiratory tract but was absent or at very low levels in nasopharyngeal swab samples. This presentation suggests that, in certain hosts or for particular variants of 2009 A(H1N1) virus, the lower respiratory tract may be the preferred site of infection.

In the majority of human influenza cases, primary influenza infection is limited to the upper respiratory tract. Significant infection of the bronchioles and alveoli as a result of direct extension from the upper airway occurs less frequently [1]. Primary viral pneumonia represents the most serious complication and is associated with the highest mortality. Pandemic influenza strains have an increased propensity to infect the lower respiratory tract and cause this most serious of influenza sequelae [1, 2]. Similarly, in highly pathogenic avian H5N1 influenza, patients more commonly present with lower respiratory tract disease without preceding upper respiratory symptoms [3].

Recently, a new pandemic swine-origin 2009 influenza A(H1N1) virus has emerged. Thus far, the majority of cases result in a mild, self-limited illness, with <5% of patients requiring hospitalization [4]. However, several studies in animal models have demonstrated an increased ability of 2009 A(H1N1), compared with seasonal influenza strains, to replicate in the lower respiratory tract and to cause pathogenic effects in lung tissue [5–7]. We present a case of severe viral pneumonia caused by 2009 A(H1N1) virus in which robust viral infection of the lower respiratory tract was detected with absent or minimal upper airway infection. This clinical presentation highlights the pathogenic potential of 2009 A(H1N1) virus and suggests that more vigilant surveillance of the lower airway may be required for severe cases.

Methods. Nasopharyngeal swab samples, endotracheal aspirates, and bronchoalveolar lavage (BAL) samples were obtained using standard collection methods from 22 June through 14 July 2009. Specimens submitted for respiratory direct fluorescent antibody (DFA) panel were tested with fluorescein-conjugated monoclonal antibodies for detection of influenza A virus, as well as a panel of common respiratory viruses (Diagnostic Hybrids). Viral cultures were performed as described elsewhere [8]. The BAL specimen obtained on day 6 was sent to the Santa Clara County Public Health Laboratory for subtyping and the California Department of Public Health for confirmatory 2009 A(H1N1) influenza real-time, reverse-transcriptase polymerase chain reaction (rRT-PCR).

As part of the retrospective laboratory investigation of this case, rRT-PCR for influenza A virus was performed on available specimens following the World Health Organization/Centers for Disease Control and Prevention April 2009 protocol [9]. The human RNase P gene (RNP) was used as an internal control for nucleic acid extraction and PCR inhibition.

Results. An 11-year-old female patient with a history of restrictive cardiomyopathy that had required heart transplantation 2 years earlier was admitted to the hospital for fever, diarrhea, and vomiting. Two weeks before hospital admission, she developed daily fevers up to 39.3°C and multiple episodes per day of emesis and loose, nonbloody stools. Her father had recently been ill with similar symptoms. One day before hospital admission, the patient developed cough and sore throat with continued fever and gastrointestinal symptoms. Her immunosuppressive regimen included cyclosporine and sirolimus.

On initial evaluation, the patient was small, thin-appearing, and in no respiratory distress. Pertinent vital signs included a temperature of 37.4°C, respiratory rate of 28 breaths per min, and 94% oxygen saturation on room air. She had no evidence of rhinorrhea, and her oropharynx was clear. Her heart examination was notable for a 1/6 systolic ejection murmur. Lung examination revealed good aeration with fine crackles at the bases. The patient's abdomen was soft, nontender, and nondistended. The patient's white blood cell count was 3400 cells/μL. A chest radiograph demonstrated a possible nodular opacity in the right upper lobe. A blood sample was obtained for culture

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Reprints or correspondence: Dr Benjamin A. Pinsky, Stanford University School of Medicine, Dept of Pathology, 300 Pasteur Dr, Lane 235, Stanford, CA 94305-5324 (bpinsky@stanford.edu).

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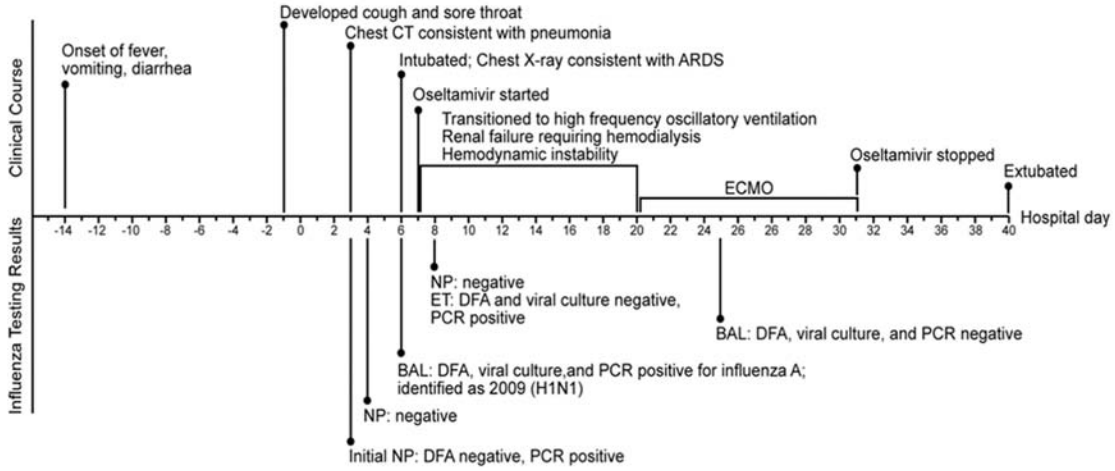

Figure 1. Time line of the patient's clinical course and key respiratory testing results. Specimens were evaluated for influenza A virus by respiratory direct fluorescent antibody (DFA) and viral culture at the time of acute illness. Real-time, reverse-transcriptase polymerase chain reaction (rRT-PCR) testing for influenza A was performed retrospectively. BAL, bronchoalveolar lavage; CT, computed tomography; ECMO, extracorporeal membrane oxygenation; ET, endotracheal aspirate; NP, nasopharyngeal swab.

and showed no growth. Multiple stool specimens were tested for bacteria, viruses, and parasites, and all test results were negative.

During the first 3 days of hospitalization, the patient experienced worsening respiratory symptoms with cough, tachypnea, and an increasing oxygen requirement. Nasopharyngeal swab samples were obtained for DFA and viral culture, which were negative. Computed tomography (CT) of the chest on hospital day 3 showed bilateral patchy consolidation with peripheral ground-glass opacities. The patient began empirical therapy with azithromycin, vancomycin, meropenem, and voriconazole. Her pulmonary status continued to decline, and the patient was transferred to the intensive care unit on day 6 and intubated for bronchoscopic examination. During the bronchoscopic examination, the patient was noted to have unremarkable mucosa and one mucous plug but no other secretions. The BAL fluid had test results that were positive for influenza A virus by DFA, and influenza A virus was later isolated from culture. Further testing identified the virus as 2009 A(H1N1) influenza. Oseltamivir therapy was started, and the patient defervesced within 24 h of therapy initiation. Bacterial cultures and cultures for acid-fast bacilli, *Legionella* species, and cytomegalovirus, as well as the results of a pneumocystis fluorescent antibody stain, were all negative.

Following bronchoscopy, the patient remained intubated. A chest radiograph had findings that were consistent with acute respiratory distress syndrome. The patient's clinical status continued to worsen. She was placed on high-frequency oscillatory ventilation and inhaled nitric oxide. She also experienced renal failure requiring continuous veno-venous hemofiltration and hypotension requiring pressor support with dopamine, epinephrine, and vasopressin. Because of worsening ventilation and oxygenation, she was placed on extracorporeal membrane oxygenation (ECMO) on day 20 and remained on ECMO for 9 days. After viral cultures from BAL specimens had negative results, oseltamivir therapy was discontinued on day 31. The patient was extubated on day 40 and has continued to show clinical improvement. A chest CT following extubation showed marked improvement of the areas of nodularity and ground glass throughout the lungs, with residual opacities in the right upper lobe and left lung base. Significant events from her hospital course and their temporal relationship with influenza A virus test results are summarized in Figure 1.

Results of DFA, viral cultures, and PCR for influenza A virus performed on respiratory specimens collected during the patient's hospitalization are shown in Table 1. The initial specimen, a nasopharyngeal swab sample collected on day 3, yielded negative DFA and positive PCR results. Notably, amplification of viral RNA in this specimen occurred at a late cycle, when the PCR reaction was near completion, despite robust amplification of the internal RNP control (Figure 2). A follow-up nasopharyngeal specimen obtained 1 day later had negative culture and PCR results. Significantly, the BAL obtained on day 6 was positive for influenza A virus by culture, DFA, and PCR and was identified as 2009 A(H1N1) virus. Treatment with oseltamivir was initiated on day 7. On day 8, concurrent nasopharyngeal and endotracheal specimens demonstrated viral RNA in the lower respiratory tract but not in the upper airway. Thereafter, endotracheal and BAL specimens continued to have positive results by PCR only until day 25. Except for the positive PCR results for the day 3 nasopharyngeal specimen, all influ-

Table 1. Influenza A Virus Test Results for Respiratory Specimens Collected during Hospitalization

Hospital day	Source	Viral culture	DFA	PCR[a]
Pretreatment				
3	NP	*ND*	Negative	Positive
4	NP	Negative	*ND*	Negative
6	BAL	Positive	Positive	Positive
Post-treatment				
8	ET	Negative	Negative	Positive
8	NP	Negative	Negative	Negative
12	ET	ND	Negative	Positive
23	ET	ND	Negative	Positive
24	BAL	Negative	Negative	Positive
25	BAL	Negative	Negative	Negative

NOTE. BAL, bronchoalveolar lavage; DFA, direct fluorescent antibody; ET, endotracheal aspirate; ND, not determined; NP, nasopharyngeal swab; PCR, polymerase chain reaction.

[a] Real-time, reverse-transcriptase PCR testing for influenza A virus was performed retrospectively.

enza A virus tests performed on nasopharyngeal specimens collected throughout the course of her illness had negative results.

Discussion. We present a case of 2009 A(H1N1) viral pneumonia with predominant lower respiratory tract infection. Although the site of initial infection was likely the upper airway, the primary site of active viral replication was the lower respiratory tract at the time of clinical presentation. First, and most significantly, only the BAL specimens obtained on day 6 demonstrated shedding of infectious viral particles by DFA and culture, in addition to viral RNA detection by PCR. Nasopharyngeal swab samples obtained before and after (on day 4 and 8, respectively) had negative results according to all tests. In contrast, the initial day 3 nasopharyngeal specimen, like all BAL and endotracheal specimens obtained after initiation of therapy, was positive by PCR only. Given the high sensitivity of DFA, compared with that of PCR, in our laboratory (87%; data not shown), the isolated PCR positivity of the day 3 nasopharyngeal specimen likely represents either very low-level viral shedding or residual viral RNA in the absence of active shedding as viral replication was transitioning to the lower respiratory tract.

Second, the amount of viral RNA present in the upper airway on the initial nasopharyngeal specimen was very low (at the lower limit of detection of the PCR assay), as indicated by the significantly delayed amplification curve (Figure 2) and the inability to detect virus by any method in subsequent nasopharyngeal specimens. Although the PCR was not quantitative per se, a delay in the threshold cycle at which significant amplification was detected was also observed in successive post-treatment endotracheal and BAL specimens until viral RNA became undetectable. Interestingly, the disappearance of detectable virus in the upper airway correlated with the worsening respiratory status of the patient. It appears that the patient's respiratory symptoms intensified just as viral shedding in the upper airway was resolving or at such a low level that it could not be reliably detected, despite significant infection in the lower respiratory tract.

The disease presentation in this case is not typical of the mild clinical syndrome described for 2009 A(H1N1) virus infection, consisting of predominantly upper airway signs and symptoms [4]. The preference of viral infection for the lower respiratory tract in this patient may be attributable to the virulence or tropism of the particular viral variant involved and/or host factors in this immunocompromised patient. Whether virus- or host-mediated, this finding highlights the pathogenic potential of 2009 A(H1N1) virus. In particular, this disease presentation provides an important clinical correlate to observations in ferret, mice, and nonhuman primate models that have demonstrated an increased ability of 2009 A(H1N1) virus to replicate in deep lung tissue [5–7].

During the 2009–2010 respiratory virus season, the 2009 A(H1N1) virus is certain to be one, if not the primary, circulating influenza virus. Although the disease may remain mild and self-limited in the majority of cases, it appears that 2009 A(H1N1) virus has the potential to act as a more virulent agent with increased capability and even a preference for lower respiratory tract infection [10]. Therefore, in patients with severe

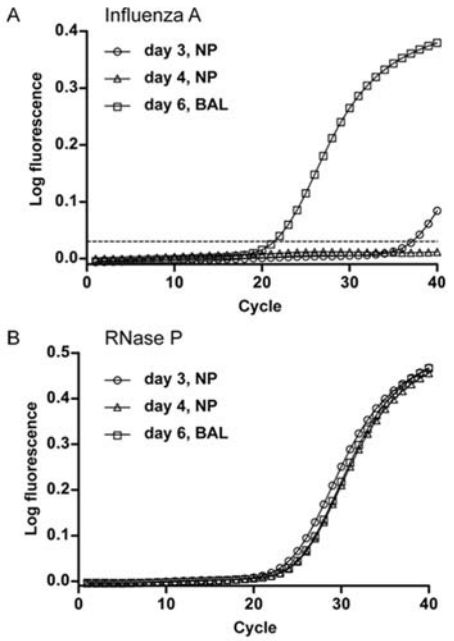

Figure 2. Polymerase chain reaction (PCR) profiles for key respiratory specimens. A comparison of the PCR amplification curves for the day 3 nasopharyngeal, day 4 nasopharyngeal, and day 6 BAL specimen is shown for *(A)* influenza A virus and *(B)* human RNase P. The amplification threshold for positive influenza A virus reactions was set at 0.03 log normalized fluorescence units, as indicated by the dashed line. BAL, bronchoalveolar lavage; NP, nasopharyngeal swab.

respiratory illness or viral pneumonia with the absence of viral detection in nasopharyngeal swab samples, empirical antiviral therapy and testing of specimens from the lower respiratory tract may be critical for patient survival and accurate diagnosis [11].

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Potential conflicts of interest. All authors: no conflicts.

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