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Fast-Track Communication

Pitfalls in Diagnosis of Pandemic (Novel) A/H1N1 2009 Influenza[▽]

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Laboratory diagnosis of influenza is important for treatment, surveillance, infection control, chemoprophylaxis, and monitoring of resistance. Rapid and sensitive nucleic acid amplification tests have widely replaced virus isolation as the reference standard (9). However, despite improvements in sensitivity and specificity, results of these tests need to be interpreted in conjunction with the history and clinical findings of the patient. False negatives may occur because of low virus quantities, poorly collected specimens, inappropriate handling, or delayed transport and technical reasons, such as the presence of viral inhibitors. Herein, we report our experience with three cases seen at Rush University Medical Center (RUMC), Chicago, IL, in which testing of nasopharyngeal swabs were negative for pandemic (H1N1) 2009 influenza virus but positive based on bronchoalveolar lavage (BAL) fluid samples (Table 1).

Patient 1. A 50-year-old male with asthma was admitted to RUMC on 6 July 2009 with a 4-day history of fevers up to 38.9°C, chills, productive cough with mild hemoptysis, and diarrhea for 1 day. He was tachycardic, with a saturation of peripheral oxygen (SpO2) of 96% in room air (RA) and a negative chest X-ray (CXR). Levofloxacin was empirically started. A polyester-tipped nasopharyngeal swab (Puritan Medical Products, Guildford, ME) in M4RT transport medium (Remel, Lenexa, KS) was collected on admission and tested negative for respiratory viruses by Luminex xTag RVP reverse transcription-PCR (RT-PCR; Luminex, Austin, TX) and Centers for Disease Control and Prevention (CDC) novel A/H1N1 RT-PCR performed at the Illinois Department of Public Health (IDPH). The patient had a chest computed tomography (CT) scan that showed bilateral upper-lobe confluent airspace opacities with multiple small lung cysts and scattered micronodules. Bronchoscopy was performed on 10 July which found copious thick clear secretions and scattered hyperemia and airway wall edema throughout both lungs. The BAL fluid sample was positive for pandemic (H1N1) 2009 influenza by both Luminex and CDC RT-PCRs. The patient improved clinically and was discharged without antiviral treatment.

Patient 2. A 25-year-old female who was 18-weeks pregnant was admitted to RUMC on 16 October 2009 with 1 week of fevers, cough, and myalgias. She had a history of asthma and thrombophilia secondary to a methylenetetrahydrofolate reductase (MTHFR) mutation, with four previous miscarriages, and was on enoxaparin for deep venous thrombosis. She was tachycardic, with an SpO2 of 97% in RA. A CXR showed a left lower lobe infiltrate, and she was started on ceftriaxone, azithromycin, and oseltamivir. Chest CT scan showed multilobar pneumonia. A nasopharyngeal swab collected on admission was negative by Luminex and CDC RT-PCRs. The patient's condition gradually deteriorated with nausea, vomiting, diarrhea, and worsening hypoxia requiring intubation on hospital day 3. Bronchoscopy done that day showed diffuse airway petechiae. The BAL fluid sample was positive for pandemic (H1N1) 2009 influenza by Luminex and CDC RT-PCRs. The patient was started on intravenous (i.v.) zanamivir; however, her condition worsened, and extracorporeal membrane oxygenation (ECMO) was initiated on 28 October. The patient expired on 30 October.

Patient 3. A 34-year-old male with obstructive sleep apnea, complex partial seizures, and partial right-frontal lobectomy was admitted to RUMC on 2 November 2009 for increased seizure frequency and 1 day of fevers up to 38.3°C. He also reported shortness of breath but had normal SpO2 of 97% in RA with negative CXR. A nasopharyngeal swab collected on admission was negative by Luminex and CDC RT-PCRs. The patient's 8-year-old son was also reportedly ill with fever and cough. The patient had intermittent low-grade fevers and a dry cough during his hospitalization with fluctuating SpO2 percentages ranging from mid-80s to low 90s. A chest CT done on 4 November showed mild bibasilar ground glass opacities. Because of persistent desaturation, CXR was repeated on 7 November and now showed new bilateral infiltrates. There were no other patients or staff with pandemic (H1N1) 2009 influenza on the same medical floor. On 8 November, the patient was intubated because of worsening hypoxemia, and a bronchoscopy and repeat nasopharyngeal swab were performed. The repeat nasopharyngeal swab was again negative for pandemic (H1N1) 2009 influenza by Luminex and CDC RT-PCRs, but the BAL fluid sample was positive by both assays. The patient was extubated on 15 November and discharged after completion of a 10-day course of peramivir.

The 2009 H1N1 influenza A pandemic has posed a number of unexpected challenges and many unanswered questions for the diagnostic microbiology laboratory. One of the fundamental issues that remains unresolved is what is the best specimen for diagnosis of influenza. Published opinions range widely regarding the diagnostic sensitivities of nasal aspirates versus washes or swabs, regular versus flocked swabs, or combined nasal and throat versus a single nasopharyngeal sample (1, 4, 6, 7). Part of the difficulty in interpreting these studies include the use of different study populations; employment of a wide variety of sampling techniques; use of different diagnostic tests, such as immunofluorescence, culture, and nucleic acid amplification tests; and selection of different groups of respiratory viruses for testing. Current CDC guidelines for laboratory diagnosis of the pandemic (H1N1) 2009 influenza virus recommend the collection of either nasopharyngeal swab, nasal aspirate, or a combined nasopharyngeal and oropharyngeal swab

TABLE 1. Characteristics of patients with positive BAL fluid samples for pandemic 2009 (H1N1) influenza

Patient no.	Age (yr)/ sex	Underlying condition(s)	Admit date/diagnosis	Date/result of:		Radiographic findings	ICU admission	Mechanical ventilation or ECMO (days)	Antiviral treatment	Length of stay/ outcome
				Nasopharyngeal swab/RT-PCR	BAL RT-PCR					
1	50/M	Asthma	6 July/viral syndrome	6 July/negative	10 July/positive	Bilateral upper lobe opacities	No	No	No	7 days/survived
2	25/F	Pregnancy, asthma, MTHFR mutation, deep venous thrombosis	16 Oct/pneumonia	16 Oct/negative	20 Oct/positive	Left lower lobe pneumonia	Yes	Mechanical ventilation (10), ECMO (2)	Oseltamivir/zanamivir i.v.	15 days/deceased
3	34/M	Complex partial seizures, obstructive sleep apnea, obesity, hyperlipidemia	2 Nov/seizures	2 Nov/negative	8 Nov/positive	Bibasilar opacities	Yes	Mechanical ventilation (5)	Oseltamivir/peramivir i.v.	18 days/survived

(3). However, there are few data on the viral kinetics of pandemic (H1N1) 2009 influenza in humans and, consequently, the optimal body site for testing.

Our report highlights the potential for false-negative results with RT-PCR performed using nasopharyngeal swabs in patients with pandemic (H1N1) 2009 influenza virus infection. Potential reasons for this include false-negative RT-PCR due to technical reasons or inadequate sampling from poorly collected nasopharyngeal swabs. However, all of our in-house RT-PCR results concurred with the CDC RT-PCR assay results, making it unlikely that these were false-negative RT-PCR results. In addition, patient 3 had a second negative nasopharyngeal swab collected simultaneously with the BAL fluid specimen, which argues against poor sampling as a likely explanation. While it remains unclear if the use of nasal aspirates may improve diagnostic yield for pandemic (H1N1) 2009 influenza compared to the use of nasopharyngeal swabs, we are aware of an unpublished case at Loyola Medical Center, Chicago, IL, in which a 26-year-old patient with severe influenza pneumonia had a negative nasal aspirate RT-PCR on admission but was later positive for pandemic (H1N1) 2009 influenza based on BAL fluid samples by both ProFlu+ (Gen-Probe Inc., San Diego, CA) and CDC RT-PCR (P. Schreckenberger, personal communication).

An alternative plausible reason for our findings is that there is more extensive viral replication in the columnar epithelial cells of the lower respiratory tract than in the squamous epithelial cells of the upper respiratory tract. In a study of ferrets infected with seasonal and pandemic (H1N1) 2009 influenza viruses, Munster et al. reported that while seasonal A/H1N1 virus replication was confined to the nasal cavity, pandemic (H1N1) 2009 influenza virus also replicated in the trachea, bronchi, and bronchioles (8). Childs et al. found that compared to seasonal human H1N1 virus and triple-reassortant swine H1N1 virus, the pandemic (H1N1) 2009 influenza virus is able to bind efficiently to both α2-6- and α2-3-linked sialyl glucan receptors (5). Binding to α2-3-linked receptors is associated with the ability of influenza viruses to cause disease in the lower respiratory tract, where there is a greater proportion of α2-3-linked sialyl glucans than α2-6-linked sialyl glucans (10).

A recent report by Blyth et al. found that 4 of 21 patients with severe pandemic (H1N1) 2009 influenza requiring intensive-care-unit admission and mechanical ventilation had negative RT-PCR specimens from the upper respiratory tract but positive bronchoscopic specimens (2). Similarly, all of our patients had evidence of lower respiratory tract disease, and two of three patients had severe influenza pneumonia requiring intensive-care-unit admission and mechanical ventilation. These findings suggest that patients with an influenza-like illness and pneumonia with negative nasal aspirate or swab RT-PCR results and no other explanation for their illness should undergo lower respiratory tract sampling for influenza RT-PCR. Establishing the diagnosis of influenza in hospitalized patients is important both for treatment and infection control purposes and may reduce orders for further diagnostic tests.

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