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Citation

Published Version
doi:10.4161/viru.22466

Permanent link
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Accessibility
Diagnosis of influenza from lower respiratory tract sampling after negative upper respiratory tract sampling

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Keywords: influenza, human, H1N1 virus, bronchoalveolar lavage, polymerase chain reaction

In this retrospective cohort study, we demonstrate that PCR-confirmed diagnoses of influenza were made solely by lower respiratory sampling in 6.9% of cases, as traditional upper respiratory tract tests were negative, indeterminate or not performed. Clinical features of these cases are presented. Clinicians should consider lower respiratory tract sampling in select cases of influenza-like illness for diagnosis.

Introduction

Clinicians are often faced with the challenge of patients presenting with an influenza-like syndrome despite negative routine influenza investigations. These investigations usually include nasal or nasopharyngeal rapid influenza screens, direct fluorescent antibody (DFA) or PCR tests. Occasionally these diagnostic tests may be falsely negative due to their low sensitivity, as in the case of many rapid-influenza tests, poor technique in specimen collection, delayed transport to the laboratory or the presence of viral inhibitors. Clinicians rely heavily on these investigations as they are readily available and guide therapeutic decisions.

Most influenza infections affect the upper respiratory tract, while lower tract infection typically represents extension from upper airways and may be diagnosed with lower respiratory sampling such as bronchoscopy. Occasionally a diagnosis of influenza is missed with upper respiratory tract sampling if pulmonary symptoms are present, and concerns have been raised regarding missing pandemic strain of H1N1 and Avian influenza A (H5N1), which have both been shown to infect the lower respiratory tract. We present data from our institution where lower respiratory tract sampling aided the diagnosis of influenza and discuss clinical features of these patients.

Methods

The Institutional Review Board at the Massachusetts General Hospital reviewed and approved this study. We performed a retrospective cohort analysis of all cases of PCR-confirmed influenza between December 2009 and April 2011 at the Massachusetts General Hospital [Simplexa™ Influenza A H1N1 (2009), Focus Diagnostics]. We identified all patients where lower respiratory sampling (induced sputum, endotracheal aspiration or bronchoscopy) was used to diagnose influenza. Only cases that were confirmed as PCR-positive were included. Using a standardized data collection form, we recorded patient demographics, influenza diagnostic testing, radiographic features, oxygenation supplementation, clinical features, accompanying comorbid conditions, and outcomes. Obesity was defined by body mass index (BMI) equal to or greater than 30. Patients were defined as immunocompromised if they were taking prednisone (or equivalent) > 15 mg per day for over 2 mo, on active chemotherapy, HIV with CD4 T cell counts less than 200 cells/ml or on other immunomodulatory medications such as biologic therapies like tumor necrosis factor antagonists.

Results

One hundred and sixteen patients were identified with PCR-confirmed influenza virus between December 2009 and April 2011. Forty-six were typed as pandemic H1N1 and 70 as seasonal influenza A. The average age was 56.6 y (range 1–95) with 60 (51.7%) females. Ninety-four patients (81%) were hospitalized and a total of 6 (5.1%) of died. Sixty-seven (57.8%) had a comorbid condition portending severe influenza. Of these 116 PCR-positive patients, 15 (12.9%) underwent lower respiratory sampling to aid in diagnosis (age range 11–81 y). Ten of these 15 patients (66.7%) were positive for influenza virus in lower respiratory samples. Of these 10, a diagnosis of influenza was made solely by lower respiratory sampling in eight cases (6.9% of total PCR positive cases), as rapid tests, nasopharyngeal DFA or PCR tests were either negative, indeterminate or not performed

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Submitted: 07/13/12; Revised: 10/03/12; Accepted: 10/05/12
http://dx.doi.org/10.4161/viru.22466
Influenza viruses initially infect the upper airways but can directly extend to the lower airways in severe cases, resulting in a viral pneumonia with significant morbidity and mortality. Patients initially present with upper respiratory symptoms, but typically deteriorate from a respiratory standpoint if lower tract symptoms develop, frequently requiring hospitalization or intensive care. Clinicians should be aware that sampling of the upper airways might not be adequate to diagnose these cases. In our series, 6.9% of PCR-documented influenza had negative upper airway sampling, and were diagnosed by either BAL, endotracheal aspiration, or induced sputum. Certain influenza strains such as Avian H5N1 virus are reported to infect lower airways, and case reports suggest that pandemic H1N1 may have a predilection for lower airways as well. Indeed, recent studies suggest that mutations to pandemic H1N1 such as D222G/N result in exclusively lower respiratory tract disease. This is concerning as it highlights the possibility for future circulating influenza strains to preferentially infect the lower respiratory tract, and clinicians should be aware that lower-tract sampling may be necessary to make or confirm an influenza diagnosis. It is difficult to ascertain if our case series supports the theory that pandemic H1N1 has a higher probability than seasonal influenza for infecting the lower respiratory tract given the descriptive and retrospective nature; however, 70% of our samples from lower tract cases were confirmed as pandemic H1N1 influenza, yet this only made up 39.7% of all PCR-type influenza diagnoses.

A weakness of this study is that it is retrospective and the prevalence of lower respiratory tract samples positive for influenza may be underestimated here. Many more patients may have lower respiratory tract disease but were not tested. We also only looked at cases of influenza that were PCR positive, and likely excluded several cases that had negative diagnostic tests. It is not clear why the five patients with lower respiratory tract symptoms who underwent BAL had negative PCR specimens. These may be related to specimen sampling, transportation or processing errors.

Several types of viral pneumonia have been diagnosed via lower respiratory tract sampling. In a primarily immunocompromised cohort of patients with pneumonia, Connoly et al. utilized BAL and culture techniques to diagnose a viral etiology in 615 out of 1,199 specimens. Eleven of these cases were influenza (nine with type A and two with type B). In addition to bronchoscopy, endotracheal aspirates in intubated patients may also be effective in diagnosing lower tract influenza. A small case series from California reported three patients with negative influenza PCR on nasopharyngeal swabs, but positive PCR for pandemic H1N1 influenza with endotracheal aspirates. Two patients in our series were diagnosed by endotracheal aspirate, both of whom had pandemic H1N1 infection. Other helpful diagnostic methods for influenza A include PCR from sputum samples, without the aid of lower respiratory tract sampling. Falsey et al. recently demonstrated the identification of respiratory viruses (primarily influenza A) in 36% of sputum samples tested with PCR compared with 23% of nasopharyngeal samples tested with PCR. Unfortunately, sputum samples are often difficult to obtain, and could only be acquired in 73% of patients.

There are several reasons why results from traditional influenza diagnostic tests may be negative in cases of influenza. Appropriate

**Table 1.** Characteristics of patients with lower respiratory specimens positive for influenza A (pandemic and seasonal)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Rapid test</th>
<th>NP DFA</th>
<th>NP PCR</th>
<th>Comorbidities</th>
<th>Lower respiratory tract sampling</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>F</td>
<td>-(1)</td>
<td>-(1)</td>
<td>n/a</td>
<td>Nil</td>
<td>ETA</td>
<td>Died</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>F</td>
<td>n/a</td>
<td>-(2)</td>
<td>n/a</td>
<td>Renal transplant</td>
<td>BAL</td>
<td>Survived</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>F</td>
<td>-(2)</td>
<td>+</td>
<td></td>
<td>Pregnant, obese</td>
<td>BAL</td>
<td>Survived</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>F</td>
<td>n/a</td>
<td>+</td>
<td>+</td>
<td>ILD</td>
<td>BAL</td>
<td>Survived</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>F</td>
<td>-(1)</td>
<td>-(1)</td>
<td>i(1)</td>
<td>Nil</td>
<td>BAL</td>
<td>Survived</td>
</tr>
<tr>
<td>6</td>
<td>26</td>
<td>M</td>
<td>-(2)</td>
<td>-(2)</td>
<td>-(1)</td>
<td>Nil</td>
<td>BAL</td>
<td>Survived</td>
</tr>
<tr>
<td>7</td>
<td>73</td>
<td>M</td>
<td>n/a</td>
<td>-(1)</td>
<td>n/a</td>
<td>ILD</td>
<td>BAL</td>
<td>Died</td>
</tr>
<tr>
<td>8</td>
<td>37</td>
<td>F</td>
<td>n/a</td>
<td>-(1)</td>
<td>n/a</td>
<td>Nil</td>
<td>ETA</td>
<td>Survived</td>
</tr>
<tr>
<td>9</td>
<td>81</td>
<td>M</td>
<td>-(1)</td>
<td>-(1)</td>
<td>-(1)</td>
<td>Nil</td>
<td>BAL</td>
<td>Survived</td>
</tr>
<tr>
<td>10</td>
<td>52</td>
<td>M</td>
<td>-(1)</td>
<td>-(1)</td>
<td>-(1)</td>
<td>HIV, asthma</td>
<td>IS</td>
<td>Survived</td>
</tr>
</tbody>
</table>

NP, nasopharyngeal; DFA, direct fluorescent antibody; i, indeterminate; BAL, bronchoalveolar lavage; ETA, endotracheal aspirate; IS, induced sputum; ILD, interstitial lung disease
nasopharyngeal sampling techniques must be employed, and such
tests may ultimately be negative due to inadequate specimen
collection. Other possibilities include low levels of viral shedding
in the nasopharynx at the time of sampling as the infection has
progressed to the lower respiratory tract.2 Animal models
demonstrate more viral replication in the trachea, bronchi and
bronchioles with pandemic H1N1 compared with seasonal
H1N1, which is restricted primarily to the nasopharynx.11
Lastly, those with risk factors for severe influenza such as obesity,
an immunocompromised state, asthma or pregnancy12 may be at
greater risk of lower respiratory tract involvement and a poor
prognosis. Future prospective studies should assess diagnostic
characteristics of influenza in relation to the time of sample
collection, risk factors for severe disease and clinical disease
progression.

Traditional nasopharyngeal diagnostic techniques may miss
cases of influenza affecting the lower respiratory tract. Clinicians
should have a high degree of suspicion in patients with lower-tract
symptoms and a syndrome compatible with influenza, particularly
in the setting of pregnancy, obesity or in immunocompromised
states. Empiric antiviral therapy is often warranted13 and sampling
of the lower tract by bronchoscopy, endotracheal aspirate or
induced sputum may yield a diagnosis.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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