Comparison of Combined Nose-Throat Swabs with Nasopharyngeal Aspirates for Detection of Pandemic Influenza A/H1N1 2009 Virus by Real-Time Reverse Transcriptase PCR

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Data assessing the diagnostic accuracies of use of different respiratory samples for the detection of the novel influenza A/H1N1 2009 virus by molecular methods are lacking. The objective of this study was to compare the sensitivity of combined nose and throat swabs (CNTS) with that of nasopharyngeal aspires (NPA). This was a prospective study of adults and children with suspected influenza. Real-time reverse transcriptase PCR testing was used for the virological diagnosis. Of the 2,473 patients included, 264 with paired CNTS and NPA were randomly selected. Novel influenza A/H1N1 virus was identified in at least one sample for 115 (43.6%) patients, the majority of them young adults. In 109 patients (94.8%) the virus was identified in the CNTS, and in 98 (85.2%) it was identified in the NPA (P = 0.02). In 93 patients (80.1%), the virus was identified in both specimens. Spearman’s rho correlation coefficient between the two methods was 0.82 (P < 0.001). There were no significant differences in accuracy between the specimens when patients were stratified according to demographic or clinical characteristics except in the case of women, in whom the sensitivity of CNTS was higher (P = 0.01). The combination of CNTS and NPA had a significantly higher sensitivity in identifying the virus than did each method alone (P = 0.02 for the comparison of the combination of both sampling methods with CNTS, and P < 0.001 for the comparison with NPA). We conclude that in patients with the novel influenza A/H1N1 virus, the diagnostic yield of CNTS is higher than that of NPA. The combination of both sampling methods increases the likelihood of diagnosing the virus.

The outbreak of the pandemic influenza A (H1N1) 2009 virus in April 2009 posed a major challenge to health services and clinicians. Factors contributing to the higher patient load and wave of hospital admissions in comparison to those during seasonal influenza (16, 17) were the rapid spread of disease and the high proportion of severe and fatal complications occurring in previously healthy young adults (12, 13). The availability of effective therapeutic measures against the virus (3) was another key factor highlighting the need for easy and sensitive methods to identify the specific viral etiology.

The diagnostic yields of different upper respiratory tract specimens for the detection of a number of viruses that cause respiratory infections have been analyzed over recent decades, although the vast majority of data have come from individuals in the pediatric age group (8) and most of the studies used viral cultures as a reference standard. Based on the results of several of these studies, nasopharyngeal aspirates (NPA) have generally been considered the specimen of choice for the identification of respiratory viruses (7, 9, 14, 15). One of the shortcomings of the use of NPA is that the procedure is unpleasant for the patient. In addition, collection of an NPA specimen requires a suction device and a skilled operator, features which make it unfeasible for widespread use in clinical practice. Collection of a nasal or throat swab is, by contrast, safer, easier, and painless, and it can be done anywhere without any additional devices. However, all these advantages might be cancelled out if the diagnostic yield of the sample was lower, since the quality of the clinical specimens is a crucial determinant for the virological diagnosis. Preliminary data suggest that the use of current molecular methods might overcome the previously observed low sensitivity seen with specimens whose collection is less invasive (5).

Knowing the accuracies of use of the different respiratory specimens when using current molecular methods is therefore crucial at the time of deciding the best diagnostic strategy. To date, no comparative studies have been performed to identify the optimal sampling procedure for the detection of the novel influenza A (H1N1) virus. This uncertainty is reflected in the World Health Organization recommendations for diagnosis of the pandemic (H1N1) 2009 virus, where it is stated that the clinical specimen that gives the best diagnostic yield remains unknown (18). The objective of this study was to compare the accuracy of use of NPA with that of a combination of nose and throat swabs for detection of the pandemic influenza A (H1N1) 2009 virus by reverse transcriptase PCR (RT-PCR).

MATERIALS AND METHODS

Study population and specimens. This population-based prospective study was carried out at the San Juan University Hospital, Alicante, Spain. All patients with suspected novel influenza A (H1N1) virus infection cared for in our institution, serving a population of 250,000 people, were included in the investigation. Patients were recruited during the outbreak of pandemic influenza A (H1N1) in Spain from July through December, 2009. During the recruitment period, respi-
The diagnostic accuracies of the sampling methods in the overall population and according to the demographic and clinical characteristics of the patients are shown in Table 2. In 109 patients (94.8%) the virus was identified in the CNTS, and in 98 cases (85.2%) it was identified in the NPA (P = 0.02). When patients were stratified according to demographic or clinical characteristics, there were no significant differences in accuracy between CNTS and NPA, with the exception of women, in whom the sensitivity of CNTS was higher than that of NPA (98% versus 83%, respectively; P = 0.01). In 93 (80.1%) of the 115 patients diagnosed as having novel influenza A (H1N1) virus infection, the PCR results identified the virus in both CNTS and NPA specimens. Spearman’s rho correlation coefficient between the two sampling methods was 0.82 (P < 0.001).

The combination of both CNTS and NPA had a significantly higher sensitivity in identifying the pandemic influenza A (H1N1) 2009 virus than each method alone (P = 0.02 for comparison of the sensitivity of CNTS with that of the combination of both tests, and P < 0.001 for comparison of the sensitivity of NPA with that of the combination of both tests). By subgroups, the combination of both CNTS and NPA had a higher sensitivity in identifying the influenza A (H1N1) virus than NPA alone in patients older than 45 years and in patients presenting with influenza-like symptoms (P < 0.05 in both cases). Such differences were not observed when comparing the combination of CNTS and NPA with CNTS alone.

**DISCUSSION**

This study shows that a combined throat and nasal sampling is superior to nasopharyngeal aspirates for the diagnosis of the novel influenza A pandemic (H1N1) 2009 virus by real-time RT-PCR. In addition, we found that the sensitivity in detecting
the virus improved with the combination of both sampling procedures. This is the first study comparing the diagnostic yields of different sampling methods during the influenza A (H1N1) 2009 pandemic. In contrast to the case for children, there is a paucity of data assessing the sensitivity of swab versus aspirate specimens for the diagnosis of influenza in adults. In a recent study, Lieberman et al. (7) evaluated three sampling methods for the identification of respiratory viruses in adults. They found that the nasopharyngeal sampling had a higher sensitivity than oropharyngeal sampling, and among nasopharyngeal specimens, nasopharyngeal washing performed better than the nasopharyngeal swab. However, for the sampling collection they used cotton swabs, which have proven to have a lower rate of recovery of respiratory pathogens than flocked swabs (2, 10). In contrast, in our study, the diagnostic yield of combined throat and nasal foam swabs was even better than that of aspirates for the diagnosis of influenza A (H1N1) virus, in addition to being easier, quicker, and less unpleasant for the patient.

Despite the fact that an NPA has typically been regarded as the specimen of choice, for children there are also data that show comparable sensitivities of nasal swabs and NPA for the detection of all major respiratory viruses except respiratory syncytial virus (4). Moreover, we processed the throat and nasal foam swabs was even better than that of aspirates for the diagnosis of influenza A (H1N1) virus, in addition to being easier, quicker, and less unpleasant for the patient.

In contrast to being easier, quicker, and less unpleasant for the patient.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. positive (n = 115)</th>
<th>Combined nasal-throat swabs</th>
<th>Nasopharyngeal aspirates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. identified</td>
<td>Sensitivity (95% confidence interval)</td>
<td>Negative predictive value (95% confidence interval)</td>
</tr>
<tr>
<td>Age group, yr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14</td>
<td>24</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>15–24</td>
<td>23</td>
<td>22</td>
<td>96 (87–100)</td>
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<tr>
<td>25–44</td>
<td>35</td>
<td>34</td>
<td>97 (92–100)</td>
</tr>
<tr>
<td>≥45</td>
<td>33</td>
<td>29</td>
<td>88 (77–99)</td>
</tr>
<tr>
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<td>55</td>
<td>50</td>
<td>91 (83–99)</td>
</tr>
<tr>
<td>Female</td>
<td>60</td>
<td>59</td>
<td>98 (95–100)*</td>
</tr>
<tr>
<td>Pregnancy</td>
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<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Clinical presentation</td>
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<td></td>
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<tr>
<td>Influenza-like illness</td>
<td>88</td>
<td>84</td>
<td>95 (91–100)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>7</td>
<td>5</td>
<td>71 (38–100)</td>
</tr>
<tr>
<td>Admitted to hospital</td>
<td>20</td>
<td>17</td>
<td>85 (69–100)</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>109</td>
<td>95 (91–99)*</td>
</tr>
</tbody>
</table>

a P = 0.01 for the comparison of combined nasal-throat swabs with nasopharyngeal aspirates.

b P = 0.02 for the comparison of combined nasal-throat swabs with nasopharyngeal aspirates.

The number of patients included in the study may have been insufficient to find differences between the sampling methods in some specific clinical situations. It could be argued that the sensitivity to detect the virus in NPA might have been affected in some cases by the freezing process. This is unlikely, since we did not observe any effect of the cryopreservation on the performance of the assay in a sample representing 10% of the specimens. Additionally, the number of positive results obtained with the aspirate was high, accounting for 85% of all positive samples, and the higher sensitivity of CNTS found in
the present study is supported by previous data (1, 4). Our study provides the first results on sensitivity of CNTS in comparison with NPA specimens in a large adult population in which a sensitive molecular biological method is used for detection of the novel influenza A (H1N1) virus.

In conclusion, when using a sensitive molecular method for detection of the novel influenza A pandemic (H1N1) 2009 virus in adult patients, the diagnostic yield of CNTS is higher than that of NPA. Since the former is also a less invasive procedure, it could be used as the method of choice in the outpatient setting to help optimize the use of virus-specific drugs. The combination of both sampling methods increases the likelihood of diagnosing the virus, and therefore this approach might be considered in patients with severe forms of the disease.

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We have no conflicts of interests.

REFERENCES