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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 aspirates (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-

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ratory samples were collected from all patients with an influenza-like illness, defined as an acute febrile illness with malaise or body aches and symptoms suggesting respiratory tract infection, including sore throat, coughing, or rhinorrhea. Patients with a chest radiograph showing interstitial or alveolar opacities were considered to have pneumonia. All samples were obtained as per standard of care for routine diagnostic testing, and informed consent was therefore not required.

Three consecutive samples were taken from each patient by trained personnel: a nose swab, a throat swab, and an NPA. The nose and throat samples were collected with different foam swabs, which were subsequently placed in the same standard sterile viral transport medium (Vircell, Granada, Spain, or Copan Diagnostics Inc., Brescia, Italy). These samples are referred to as combined nose-throat swabs (CNTS). Nasal swab collection was performed by inserting the swab into one nostril until reaching resistance and rotating it gently. All NPA were collected according to a standard protocol. Briefly, a disposable delicate tube (Izasa Distribuciones Técnicas, S.A., Barcelona, Spain) was inserted into one of the patient's nostrils to a depth of 5 to 8 cm, and 3 to 5 ml of a sterile physiological saline solution were instilled while the patient was lying down. The instilled fluid was subsequently suctioned out and emptied into the same standard sterile viral transport medium (Vircell, Granada, Spain, or Copan Diagnostics Inc., Brescia, Italy). All the specimens were transported immediately to the laboratory or, when this was not possible, were refrigerated and delivered to the laboratory within 24 to 48 h of collection. Combined nose-throat swabs were tested upon reception at the laboratory. Nasopharyngeal aspirates were frozen and kept at −80°C for further analysis. We previously ensured that cryopreservation had no effect on the performance of the assay used for detection of the novel influenza A (H1N1) 2009 virus by testing 25 paired fresh and frozen specimens.

Detection of novel influenza A (H1N1) virus. Nucleic acids were extracted with the MagNA Pure Compact instrument (Roche Applied Science, Mannheim, Germany) with Nucleic Acid Isolation Kit I following a previously described procedure (11). Briefly, a 400-μl respiratory specimen volume was used for extraction without a prior centrifugation step, and an elution volume of 50 μl was selected. Two real-time RT-PCR (rRT-PCR) assays were performed for influenza A and B viruses and for influenza A virus subtype confirmation. The first rRT-PCR was performed with the artus_Influenza LC rRT-PCR kit (Qiagen, Hilden, Germany), a ready-to-use system for the detection of influenza virus-specific RNA using PCR in the LightCycler instrument and the reagent manufacturer's recommended cycling parameters. A second rRT-PCR was performed on influenza A/B virus-positive samples with the Real-Time Ready Influenza A/H1N1 Detection Set (Roche Applied Science). The kit contains premixed primers and hydrolysis probes for detection of the influenza A virus matrix protein 2 and hemagglutinin H1 (Mexico) genes. All tests were performed in accordance with the manufacturer's instructions.

Patients were defined as positive for influenza A (H1N1) virus if the virus was identified by rRT-PCR in any of the samples obtained.

Statistical analysis. Sensitivities, specificities, and positive and negative predictive values with 95% confidence intervals of the samples were calculated from two-by-two contingency tables. Rates between samples were compared with the chi square or Fisher's exact test where appropriate. The bivariate Spearman correlation test was used to examine the strength of association between CNTS and NPA. Statistical significance was set at a P value of <0.05. Data were analyzed using the SPSS software package version 12.0 (SPSS Inc., Chicago, IL).

RESULTS

During the study period, CNTS and NPA samples were collected from 2,473 patients. For the present analysis, 264 patients with paired CNTS and NPA specimens available were randomly selected.

The main demographic characteristics of the 264 patients are shown in Table 1. There were 135 males (51.1%) and 129 females (48.9%), with a median age of 38 years (range, 1 month to 93 years). In 115 patients (43.6%), influenza A (H1N1) virus was identified in at least one sample. The majority of these patients were young adults (73.9% were ≥18 years, and 61.2% of them were under 45 years old), and the most frequent clinical syndrome at presentation was an influenza-like illness (76.5%).

TABLE 1. Baseline characteristics of patients with suspected influenza and novel influenza A (H1N1)

Characteristic	No. (%) of patients with:	
	Suspected influenza (n = 264)	Novel influenza A (H1N1) (n = 115)
Median age, yr (interquartile range)	38 (21.5–67.5)	31 (17–47)
Age group, yr		
0–14	45 (17)	24 (20.9)
15–24	34 (12.9)	23 (20.0)
25–44	64 (24.2)	35 (30.4)
≥45	114 (43.2)	33 (28.7)
Male	135 (51.1)	55 (47.8)
Female	129 (48.9)	60 (52.2)
Pregnant women	4 (1.5)	1 (0.9)
Clinical presentation		
Influenza-like illness	158 (59.8)	88 (76.5)
Pneumonia	49 (18.6)	7 (6.1)
Site of management		
Outpatient	180 (68.2)	95 (82.6)
Admitted to hospital	84 (31.8)	20 (17.4)

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

TABLE 2. Diagnostic accuracies of combined nose-throat swabs and nasopharyngeal aspirates according to patient characteristics

Characteristic	No. positive (n = 115)	Combined nasal-throat swabs			Nasopharyngeal aspirates		
		No. identified	Sensitivity (95% confidence interval)	Negative predictive value (95% confidence interval)	No. identified	Sensitivity (95% confidence interval)	Negative predictive value (95% confidence interval)
Age group, yr							
0–14	24	24	100	100	21	88 (74–100)	88 (74–100)
15–24	23	22	96 (87–100)	95 (87–100)	20	87 (73–100)	88 (74–100)
25–44	35	34	97 (92–100)	97 (90–100)	31	89 (78–99)	88 (77–99)
≥45	33	29	88 (77–99)	95 (91–100)	26	79 (65–93)	92 (86–98)
Male	55	50	91 (83–99)	94 (89–99)	48	87 (78–96)	92 (86–98)
Female	60	59	98 (95–100)[a]	99 (96–100)	50	83 (74–93)	87 (80–95)
Pregnancy	1	1	100	100	1	100	100
Clinical presentation							
Influenza-like illness	88	84	95 (91–100)	95 (89–100)	79	90 (83–96)	89 (82–96)
Pneumonia	7	5	71 (38–100)	95 (89–100)	5	71 (38–100)	95 (89–100)
Admitted to hospital	20	17	85 (69–100)	96 (91–100)	17	85 (69–100)	96 (91–100)
Total	115	109	95 (91–99)[b]	96 (93–99)	98	85 (79–92)	90 (85–94)

[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 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 samples in a single container as a single specimen. This may have provided a higher diagnostic yield than that of each single sampling method. For respiratory bacteria, mixing of upper respiratory samples has been shown to augment the sensitivity of cultures in adults, while decreasing costs (6). In children, CNTS have been shown to have a sensitivity comparable to that of NPA (5) for respiratory virus identification, and in adults, pooled throat and nasal specimens provided an even higher diagnostic yield for coronavirus detection than NPA during the severe acute respiratory syndrome (SARS) epidemic in 2003 (1). Whether the sensitivity of pooled throat and nasal specimens is different from that of nasopharyngeal swabs (one of the alternative methods used for the diagnosis of respiratory viruses) cannot be determined from the study, since the latter procedure was not carried out, although it has usually performed worse than NPA in children (8).

There were no differences between the CNTS and NPA methods when patients were stratified according to demographic characteristics and clinical data, with the exception of the subgroup of women, in whom the sensitivity of NPA was significantly lower than that of CNTS. The significance of this finding is uncertain, and it should be confirmed in other studies. There also were no differences between swabs and aspirates in patients with a more severe clinical course, such as those developing pneumonia, although the number of patients included in this group was low. However, interestingly, the likelihood of achieving a diagnosis was higher when the two sampling methods were combined. This finding might be useful under certain clinical conditions, such as patients requiring hospital admission or developing a lower respiratory tract infection. Although the low number of patients included in both groups did not allow any difference between the combination of all versus the isolated sampling methods to be detected, the combination of swabs and aspirate might be advisable in cases with a higher severity of disease.

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.

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