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NOTES

Comparative Study of Nasopharyngeal Aspirate and Nasal Swab Specimens for Diagnosis of Acute Viral Respiratory Infection[▽]

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Paired nasopharyngeal aspirate (NPA) and nasal swab (NS) samples from 475 children hospitalized for acute respiratory infection were studied for the detection of influenza virus, parainfluenza virus, respiratory syncytial virus, and adenovirus by immunofluorescence test, viral culture, and multiplex PCR assay. The overall sensitivity of viral detection with NPA specimens was higher than that obtained with NS specimens.

Acute respiratory infection (ARI) is the most common cause of hospital admission for children in Hong Kong, and viral etiologies have been shown to play an important role (8, 10). ARIs caused by different viruses may require different infection control measures and treatments, yet they cannot be reliably distinguished on clinical grounds alone.

The standard investigational methods for suspected viral ARIs in Hong Kong public hospitals include an immunofluorescence (IF) test and viral culture of nasopharyngeal aspirate (NPA) specimens. The IF test detects influenza A and B viruses; parainfluenza type 1, 2, and 3 viruses; respiratory syncytial virus (RSV); and adenoviruses. The procedure of obtaining an NPA specimen is uncomfortable and often frightening for young children. It is also unpleasant for the medical staff, who have to carry out the procedure with a struggling, crying, and coughing child. In clinical practice, the optimal sampling methods must be balanced with patient comfort, cost, effectiveness, and risk to others. Previous reports have shown that nasal swabs (NS) are as good as NPAs for the detection of influenza virus by the IF test or enzyme immunoassay (2, 4). The sensitivity obtained by using NS and the IF test for the diagnosis of RSV infection, however, is controversial (3, 4, 7). It is possible that the best type of sample and the best collection site may depend on both the specific virus and the nature of the diagnostic test (1; http://www.cdc.gov/flu/professionals/infectioncontrol/maskguidance.htm).

The present study was undertaken with children under 5 years of age to compare the usefulness of NS and NPAs for the detection of influenza A and B viruses; parainfluenza type 1, 2, and 3 viruses; RSV; and adenoviruses by three different diagnostic tests (IF test, culture, and PCR assay).

Patients. This prospective study systematically sampled children (≤5 years of age) admitted with ARIs to a university-affiliated general hospital (Prince of Wales Hospital) from November 2005 to October 2006. Ethical approval was obtained from the University Clinical Research Ethics Committee. All eligible children admitted on Monday and Tuesday with a parent's consent were included. The diagnostic criteria for ARIs were sudden onset (<36 h) of one or more of the following symptoms and signs: rhinorrhea, cough, sore throat, earache, hoarseness, stridor, wheeze, and dyspnea with or without fever.

Sample collection and investigations. NS and NPA samples were taken by trained nurses. For NS, a Dacron swab with a plastic shaft (Copan, Italy) was placed 1 to 1.5 cm into the nostril and rotated three times against the surface of the nasal cavity. For NPAs, the catheter was inserted into the opposite nostril to a depth of 5 to 7 cm and drawn back while applying gentle suction with an electric suction device (3). Both specimens were placed into viral transport medium and kept at 4 to 10°C until further processing. All specimens were subjected to respiratory virus detection by the IF test, conventional virus culture, and multiplex nested PCR as described previously (5). Briefly, the direct IF test was used to detect influenza A and B viruses, parainfluenza virus types 1 to 3, RSV, and adenovirus with specific antibodies (Chemicon, Temecula, CA). Virus culture was accomplished with HEp-2, MDCK, and LLC-MK_2 cell monolayers, and virus growth was confirmed by IF test. Viral RNA and DNA were extracted with the QIAamp MinElute Virus Spin kit (Qiagen) for the multiplex PCR test as previously described (5). Briefly, four groups of multiplex nested fast PCR assays were used in this study: (i) influenza A and B virus group-specific and subtype H1-, H3-, H5-specific primers; (ii) parainfluenza virus types 1, 2, 3, and 4; (iii) RSV A and B; and (iv) adenovirus. Besides the above-mentioned pathogens, group 3 also included rhinovirus and enterovirus and group 4 included *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumophila*. As these additional pathogens were only investigated by the PCR method and no comparison could be made with the IF test or the culture

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TABLE 1. Viral identification in 475 paired NPA and NS samples from hospitalized children with ARI tested by IF, culture, and PCR

Virus, no. of samples positive[c] (% of total[a]), and test method	No. of tests positive[c] with either or both samples (% of total[a])	No. of tests				Agreement between NPA and NS by Cohen's kappa[b] (95% CI)[d]
		NPA^+ NS^-	NPA^- NS^+	NPA^+ NS^+	NPA^- NS^-	
Adenovirus, 29 (6.1)						
IF	12 (2.5)	2	2	8	17	0.80 (0.60–0.99)
Culture	27 (5.7)	2	2	23	2	0.92 (0.83–1.00)
PCR	25 (5.3)	5	0	20	4	0.88 (0.78–0.98)
Influenza A virus, 40 (8.4)						
IF	22 (4.6)	6	0	16	18	0.84 (0.71–0.96)
Culture	29 (6.1)	3	4	22	11	0.86 (0.75–0.96)
PCR	37 (7.8)	4	7	26	3	0.81 (0.71–0.92)
Influenza B virus, 18 (3.8)						
IF	12 (2.5)	9	1	2	6	0.28 (0.01–0.59)
Culture	14 (2.9)	2	0	12	4	0.92 (0.81–1.00)
PCR	18 (3.8)	2	2	14	0	0.87 (0.75–1.00)
Parainfluenza virus types 1–4, 49 (10.3)						
IF	26 (5.5)	9	2	15	23	0.72 (0.56–0.88)
Culture	31 (6.5)	11	2	18	18	0.72 (0.58–0.87)
PCR	49 (10.3)	9	3	37	0	0.85 (0.76–0.93)
RSV, 47 (9.9)						
IF	44 (9.3)	12	3	29	3	0.78 (0.67–0.89)
Culture	40 (8.4)	12	2	26	7	0.77 (0.66–0.89)
PCR	40 (8.4)	10	2	28	7	0.81 (0.71–0.91)
Any of above, 183 (38.5)						
IF	116 (24.4)	38	8	70	67	0.69 (0.61–0.78)
Culture	141 (30.0)	30	10	101	42	0.79 (0.73–0.85)
PCR	169 (35.6)	30	14	125	14	0.80 (0.74–0.86)

[a] Total of 475 cases.
[b] Cohen's kappa: >0.8, almost perfect agreement; 0.6 to 0.8, substantial agreement; 0.4 to 0.6 moderate agreement; <0.4, poor agreement.
[c] Positive for any specimen by any method.
[d] CI, confidence interval.

method, the details are not presented here. Stringent precautions were followed to prevent cross-contamination (5).

Statistical analyses. The agreement of the results obtained by the two different sample collection methods (NPA and NS) was assessed with Cohen's kappa test (6) separately for each viral identification method (IF test, culture, and PCR assay).

For comparison of the sensitivities obtained with NPAs and NS, a patient was considered to have a viral infection if the virus was isolated in cell culture from either an NPA or an NS specimen and/or any specimen was positive at least by the other two methods and/or both specimens were positive by any method. The sensitivities and specificities obtained with NPA and NS specimens were compared by using the chi-square test for homogeneity of proportions.

The adequacy of specimen collection was evaluated by categorizing the quality of the NPA or NS specimens by the number of columnar cells as being high (>50 cells per high-power view), medium (10 to 50 cells), or low (<10 cells). For NS specimens, an additional analysis was performed according to the total cell count (both columnar and squamous cells). The association between the quality of the specimens and the positive virus identification rate was performed with the exact chi-square test. A value of $P < 0.05$ was taken to be significant.

Paired samples were collected between November 2005 and October 2006 from 475 children under 5 years old (253 boys, 222 girls) with a mean age of 23.8 months (standard deviation, 17 months). The study sample represented 20% of the 2,404 children under 5 years of age hospitalized for acute respiratory disease during this period. The total positive yields of adenovirus, influenza A and B viruses; parainfluenza virus types 1, 2, and 3; and RSV from either or both specimens were 24, 30, and 35%, respectively, for the IF test, culture, and the PCR assay. The agreement of virus detection results between NPA and NS specimens by the three different methods, in general, was excellent (Cohen's kappa, >0.8) or substantial (0.6 to 0.8) (6), except when the IF method was used for the detection of influenza B virus (Table 1).

The overall sensitivity of viral detection with NPA specimens was significantly higher than that obtained with NS. The sensitivity was 0.67, 0.82, and 0.91, respectively, for the IF test, culture, and the PCR method with NPA specimens. The corresponding values for NS were 0.48, 0.69, and 0.81 (all $P < 0.01$) (Table 2). When the IF method was used, the sensitivity obtained with NPA specimens was significantly higher than that obtained with NS for influenza B virus and RSV, but this was not the case for the other three viruses. When culture was used, the sensitivity obtained with the NPA specimens was significantly higher for parainfluenza virus and RSV than that obtained with the NS specimens. Notably, when PCR was used,

TABLE 2. Comparison of the sensitivity and specificity of NPA and NS specimens for the detection of respiratory viruses by IF, culture, and PCR

Virus (no. of samples positive[a] by "gold standard") and test	NPA		NS		*P* value[b]	*P* value[c]
	Sensitivity (95% CI)[d]	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)		
Adenovirus (28)						
IF	0.36 (0.19–0.56)	1.00 (0.99–1.00)	0.36 (0.19–0.56)	1.00 (0.99–1.00)	0.999	0.999
Culture	0.89 (0.72–0.98)	1.00 (0.99–1.00)	0.89 (0.72–0.98)	1.00 (0.99–1.00)	0.999	0.999
PCR	0.86 (0.67–0.96)	1.00 (0.99–1.00)	0.71 (0.51–0.87)	1.00 (0.99–1.00)	0.191	0.317
Influenza A virus (32)						
IF	0.69 (0.50–0.84)	1.00 (0.99–1.00)	0.50 (0.32–0.68)	1.00 (0.99–1.00)	0.124	0.999
Culture	0.78 (0.60–0.91)	1.00 (0.99–1.00)	0.81 (0.64–0.93)	1.00 (0.99–1.00)	0.757	0.999
PCR	0.88 (0.71–0.96)	1.00 (0.98–1.00)	0.84 (0.67–0.95)	0.99 (0.97–1.00)	0.719	0.155
Influenza B virus (16)						
IF	0.69 (0.41–0.89)	1.00 (0.99–1.00)	0.19 (0.04–0.46)	1.00 (0.99–1.00)	0.003	0.999
Culture	0.88 (0.62–0.98)	1.00 (0.99–1.00)	0.75 (0.48–0.93)	1.00 (0.99–1.00)	0.367	0.999
PCR	1.00 (0.79–1.00)	1.00 (0.99–1.00)	0.88 (0.62–0.98)	1.00 (0.98–1.00)	0.151	0.157
Parainfluenza virus (41)						
IF	0.59 (0.42–0.74)	1.00 (0.99–1.00)	0.41 (0.26–0.58)	1.00 (0.99–1.00)	0.121	0.999
Culture	0.71 (0.54–0.84)	1.00 (0.99–1.00)	0.49 (0.33–0.65)	1.00 (0.99–1.00)	0.040	0.999
PCR	0.95 (0.83–0.99)	0.98 (0.97–0.99)	0.95 (0.83–0.99)	1.00 (0.99–1.00)	0.999	0.033
RSV (43)						
IF	0.93 (0.81–0.99)	1.00 (0.99–1.00)	0.70 (0.54–0.83)	1.00 (0.98–1.00)	0.005	0.563
Culture	0.88 (0.75–0.96)	1.00 (0.99–1.00)	0.65 (0.49–0.79)	1.00 (0.99–1.00)	0.001	0.999
PCR	0.88 (0.75–0.96)	1.00 (0.99–1.00)	0.67 (0.51–0.81)	1.00 (0.99–1.00)	0.018	0.317
Any of above (160)						
IF	0.67 (0.59–0.74)	1.00 (0.98–1.00)	0.48 (0.40–0.56)	0.99 (0.98–1.00)	<0.001	0.563
Culture	0.82 (0.75–0.88)	1.00 (0.99–1.00)	0.69 (0.62–0.76)	1.00 (0.99–1.00)	<0.009	0.999
PCR	0.91 (0.86–0.95)	0.97 (0.95–0.99)	0.81 (0.74–0.87)	0.97 (0.95–0.99)	0.009	0.806

[a] A patient was considered to have a viral infection if (i) the virus was isolated in cell culture from either an NPA or an NS specimen and/or (ii) any specimen was positive at least by the other two methods and/or (iii) both specimens were positive by any method.
[b] *P* value for the comparison of sensitivity between NPA and NS for detection of respiratory viruses.
[c] *P* value for the comparison of specificity between NPA and NS for detection of respiratory viruses.
[d] CI, confidence interval.

the sensitivity obtained with NPAs was significantly higher than that obtained with NS only for RSV.

There was no association demonstrated between the number of columnar and squamous cells in the specimens and positive virus identification, indicating that the number of cells in these specimens had most likely reached the threshold for all three virology tests.

Previous studies comparing NPA and NS specimens have used viral culture, an IF test, or an enzyme immunoassay, and all except one have focused on RSV or influenza virus (2–4, 7, 9). Our study used three different laboratory methods (IF test, culture, and PCR assay) to look at five important respiratory viruses. The strength of this study is that we applied all of the techniques used to all of the samples in parallel instead of using a discrepant analysis strategy of one test at a time. When the IF method was used, the NS specimen results exhibited a significant reduction in the sensitivity of detecting influenza B virus and RSV. However, there were no significant differences in sensitivity for the two different methods of specimen collection when the more sensitive PCR method was used to detect the viruses studied, with the sole exception of RSV.

In clinical practice, the presence of rhinorrhea, wheezing, and interstitial or lobar consolidation on the chest radiograph can be of help in differentiating viral respiratory tract infections from common bacterial pathogens, but a more definitive diagnosis depends on the demonstration of the virus in respiratory secretions. With the increasing concern about emerging or reemerging respiratory viral infections, especially with the alarming morbidity and mortality associated with the avian H5N1 influenza virus and severe acute respiratory syndrome, effective and early diagnosis of the viral etiology of an ARI has become a priority, both for providing more specific and timely treatment and for optimal infection control. This is especially true for the H5N1 influenza virus, when specific treatments (e.g., neuraminidase inhibitors) and early diagnosis can have a major impact on both the individual patient and the global community.

Among the diagnostic modes, viral culture typically has a high yield and has traditionally been the reference standard for diagnosis. However, this method is frequently irrelevant to practical clinical decision making because of the long lag time to obtain results. The IF method with NPA specimens is widely used in both public and private hospitals in Hong Kong and can provide a diagnosis of RSV or influenza virus infection within a few hours. However, in outpatient clinical settings, collection of NPA specimens is usually not possible because of the requirements of better-trained personnel, suction devices, and a designated area with a functional ventilation system for

infection control. PCR methods are very sensitive and can yield results within a clinically relevant time frame but are costly and technically demanding, precluding their use in most settings. The laboratory costs for culture, the IF test, and the PCR assay vary with different settings, e.g., depending on the number of specimens that are included in each run. The turnaround time for the IF test is typically 2 to 3 h, whereas 1 working day is required for the fast PCR method used in this study. Ideally, a single-round PCR assay is preferred as it is less prone to cross-contamination compared to a nested PCR assay. However, our previous study has shown that only 30% of positive samples were detectable by a single-round PCR assay (5). Precautions to minimize cross-contamination is crucial when implementing this multiplex nested PCR as a routine diagnostic service.

This study demonstrates that NS is an inadequate sample if diagnostic IF tests alone are used for the detection of the common respiratory viruses described in this study, but if PCR methods are used, diagnostic yields are comparable to the results obtained with NPA specimens for all of the viruses studied except RSV. Multiplex PCR has the additional potential benefit of providing a more comprehensive assessment of other important respiratory viruses and atypical bacteria that are fastidious or dangerous to grow. The ideal surveillance of pandemic influenza would include the systematic routine collection of less intrusive NS samples for multiplex PCR testing from all ARI patients in both public and private settings. However, further work is needed to appropriately verify and validate the most suitable molecular assay for each specific pathogen and economic factors also need to be taken into consideration to determine whether such a strategy is ultimately feasible.

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