Diagnostic Value of Nasopharyngeal Aspirates in Children with Lower Respiratory Tract Infections

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Abstract

Background: The accuracy of nasopharyngeal aspirate (NPA) specimens in detecting lower respiratory pathogens remains controversial. The objective of this study was to evaluate the diagnostic accuracy of aspirates (NPAs) specimen in lower respiratory tract infections (LRTIs) in children.

Methods: The prospective study was designed to collect the data of paired NPAs and bronchoalveolar lavage fluids from children with acute LRTIs from January 2013 to December 2015. All specimens were subjected to pathogen detection: bacterial detection by culture, *Mycoplasma pneumoniae* (Mp) detection by polymerase chain reaction assay and virus (influenza A and B viruses, parainfluenza virus [PIV] Types 1 and 3, respiratory syncytial virus, and adenovirus) detection by immunofluorescence assay. The diagnostic accuracy analysis of NPAs was stratified by age ≤3 years (*n* = 194) and >3 years (*n* = 294).

Results: We collected paired specimens from 488 children. The positive rate of pathogen was 61.6%. For *Streptococcus pneumoniae*, NPA culture had the specificity of 89.9% and negative predictive value of 100% in age ≤3 years, the specificity of 97.2% and negative predictive value of 98.9% in age >3 years. For Mp, the positive predictive values of NPA was 77.4% in children ≤3 years, and 89.1% in children >3 years. For PIV III, NPA specimen had the specificity of 99.8% and negative predictive value of 96.5% in children ≤3 years. For adenovirus, NPA had the specificity of 97.8% and negative predictive value of 98.4% in age ≤3 years, the specificity of 98.9% and negative predictive value of 99.3% in age >3 years.

Conclusions: NPAs are less invasive diagnostic respiratory specimens, a negative NPA result is helpful in "rule out" lower airway infection; however, a positive result does not reliably "rule in" the presence of pathogens.

Key words: Bronchoalveolar Lavage; Diagnostic Accuracy; Lower Respiratory Tract Infection; Nasopharyngeal Aspirate

INTRODUCTION

Globally, lower respiratory tract infections (LRTIs) are a common cause of death in children aged <5 years.[1] LRTIs caused by different microorganisms might require different infection control measures and treatments; however, they cannot be reliably distinguished on clinical grounds alone. In general, for children, the standard investigational method for suspected pathogens of LRTIs is detecting organisms in nasopharyngeal aspirate (NPA) specimens, with the results influencing therapies such as antibiotic choices.[2-4] Nonetheless, the accuracy of NPA specimens in detecting lower respiratory pathogens remains controversial.

The purpose of this study was to evaluate the diagnostic values of NPA specimens in children with LRTIs. In this study, we prospectively compared detectable rates of pathogens between NPA and bronchoalveolar lavage fluid (BALF) specimens from children with LRTIs.

METHODS

Subjects

This was a prospective study. Children with acute severe LRTIs admitted to the Children's Hospital of Fudan University from January 2013 to December 2015 were

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enrolled. The diagnostic criteria for acute severe LRTIs were a severe radiological findings including large effusion, pulmonary consolidation, atelectasis, with or without pleural effusion who needed bronchoscopy treatment, accompanied by a sudden onset (<1 week) of one or more of the following symptoms and signs: cough, wheeze, and dyspnea with or without fever. Consent for participation was obtained. The study was approved by the hospital's ethics committee.

Nasopharyngeal aspirate and bronchoalveolar lavage fluid specimen collection

Paired NPA and BALF samples were collected by trained clinical staff in accordance with a standard protocol, and NPA specimens were collected before BALF specimens. For NPA specimens, a catheter was inserted into the opposite nostril to a depth of 5–7 cm and drawn back while applying a gentle suction with an electric suction device.[5] Flexible bronchoscopy was performed through the transnasal route under conscious intravenous sedation with midazolam. Topical anesthesia of the larynx, trachea, and carina was achieved as needed with 2% lidocaine. Use of the suction channel was avoided until the tip of the bronchoscope approached just below the carina. The bronchoscope was wedged in the lesion's segment or lobe, and the lavage was performed with three aliquots of nonbacteriostatic saline, 1 ml/kg each, with a suction pressure of 100 mmHg. All specimens were transported and processed according to the requirements of the laboratory.

Microbiology

The collected specimens were subjected to common microorganism detection: bacterial detection by the conventional bacterial culture, *Mycoplasma pneumoniae* (Mp) detection by polymerase chain reaction (PCR) assay (Shanghai Shen Yousheng Biotech, Shanghai, China) and virus detection by Chemicon direct immunofluorescence assay (DIA). The hospital's clinical virology laboratory has routinely conducted DIA testing on respiratory specimens for seven respiratory viruses: influenza A and B viruses, parainfluenza virus types 1–3 (PIV I, PIV II, PIV III), respiratory syncytial virus (RSV), and adenovirus.

Statistical analysis

Continuous variables were described as mean ± standard deviation (SD) or median (range) and compared using Student's *t*-test or Mann-Whitney *U*-test according to its distribution. The difference of positive rate between the two sample collection methods (NPA and BALF) was compared using McNemar test. The agreement of the results obtained by the two different sample collection methods (NPA and BALF) was assessed with Cohen's kappa test for each identified microorganism.[6] The sensitivity, specificity, and predictive values of NPA specimens were calculated from 2 × 2 tables, with confidence intervals calculated using the binominal exact method. SPSS version 16.0 (SPSS Inc., Chicago, IL,USA) was used for data processing and $P < 0.05$ was considered statistically significant.

RESULTS

Subject characteristics

We enrolled 488 children, the median age was 5 years (range: 2 months–17 years). Three hundred were boys and 188 were girls. The median age of girls (5 years, range: 2 months–17 years) was older than the median age of boys (4 years, range: 2 months–15 years) ($P = 0.002$).

Lower respiratory tract microbiology

BALF yielded organisms in 301 children (61.6%), including *Streptococcus pneumoniae* (Sp), Group A streptococci (Ga), *Haemophilus influenzae* (Hi), *Staphylococcus aureus* (Sa), *Pseudomonas aeruginosa* (Pa), *Acinetobacter baumani* (Ab), *Escherichia coli* (Ec), *Enterobacter aerogenes* (Eb), Mp, PIV I, PIV III, and adenovirus. Positive rates of bacteria, Mp and virus in BALF specimens were 5.3%, 55.9%, 4.7%, respectively. Pathogens of bacteria sequentially were Sp (10, 2.1%), Ab (5, 1.0%), Pa (4, 0.8%), Hi (3, 0.6%), Ec (2, 0.4%), Eb (2, 0.4%), Ga (1, 0.2%), and Sa (1, 0.2%). Pathogens of viruses sequentially were PIV III (11, 2.3%), adenovirus (11, 2.2%), and PIV I (1, 0.2%). In 15 children (3.1%), more than one organism was isolated concurrently from BALF: Bacteria and Mp in six children, Mp and virus in four children, bacteria and virus in four children, and all three organisms in one child.

Because age affects the prevalence of lower airway pathogens, diagnostic accuracy was assessed for two age categories: ≤3 years, and >3 years. The prevalence of Mp infection appeared higher in both age ranges, while the prevalence of bacteria and virus infection decreased with age [Figure 1].

Diagnostic accuracy of nasopharyngeal aspirate test

As low prevalence might influence positive predictive value of screening test greatly, the diagnostic accuracy of NPA was not assessed for Ab, Pa, Hi, Ec, Eb, Ga, Sa, and PIV I infection.

For Sp, in children with age ≤3 years, there was a significant difference in the positive rate of Sp infection between NPA and BALF specimens ($P < 0.0001$) [Table 1]. NPA cultures
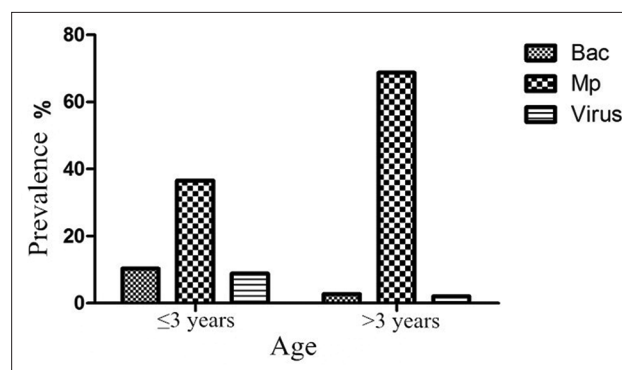

Figure 1: Age-specific prevalence of pathogens isolated from bronchoalveolar lavage fluid in 488 children with lower respiratory tract infections. Bac: Bacterial; Mp: *Mycoplasma pneumoniae*.

had the specificity of 89.9% and negative predictive value of 100% in age ≤3 years. In children with age >3 years, there was no significant difference in the positive rate of Sp infection between NPA and BALF specimens (*P* = 0.22) [Table 1]. NPA cultures had the specificity of 97.2% and negative predictive value of 98.9% in age >3 years. The agreement of Sp detection results between NPA and BALF specimens were just fair [Table 1, Cohen's kappa = 0.3 or 0.2, respectively].

For Mp, there was no significant difference in the positive rate of Mp infection between NPA and BALF specimens in children ≤3 years (*P* = 0.18), while there was a significant difference in children >3 years (*P* = 0.02). The agreement of Mp detection between NPA and BALF specimens was substantial [Table 1, Cohen's kappa = 0.60].

For PIV III, in children ≤3 years, there was no significant difference in the positive rate between NPA and BALF specimens [Table 1]. The NPA specimen had the specificity of 99.8% and negative predictive value of 96.5%, while with the sensitivity of 33.3% and positive predictive value of 15%. The agreement of PIV III detection results between NPA and BALF specimens was very poor [Table 1, Cohen's kappa = 0.1].

For adenovirus, there was no significant difference in the positive rate of adenovirus infection between NPA and BALF specimens [Table 1]. NPA specimen had the specificity of 97.8% and negative predictive value of 98.4% in age ≤3 years, the specificity of 98.9% and negative predictive value of 99.3% in age >3 years, while the sensitivity and positive predictive values decreased with ages [Table 1]. The agreement of adenovirus detection between NPA and BALF specimens was fair in all age ranges [Table 1].

DISCUSSION

Among the diagnostic modes, the test of the specimen from lower respiratory tract has traditionally been the reference

Table 1: Diagnostic accuracy of nasopharyngeal aspirates relative to BALFs for two age categories

Items	≤3 years (*n* = 194)	>3 years (*n* = 294)
Streptococcus pneumoniae		
Positive rate in BALF, *n* (%)	5 (2.6)	5 (1.7)
Positive rate in NPA, *n* (%)	24 (12.3)	10 (3.4)
P	<0.001	0.22
Sensitivity, % (95% *CI*)	100 (56.5–100.0)	40 (11.7–76.9)
Specificity, % (95% *CI*)	89.9 (84.8–93.4)	97.2 (94.6–98.6)
Positive predictive value, % (95% *CI*)	20.8 (9.2–40.4)	20 (5.6–50.9)
Negative predictive value, % (95% *CI*)	100 (97.8–100.0)	98.9 (96.9–99.6)
Cohen's kappa (95% *CI*)	0.3 (0.2−0.4)	0.2 (0.1−0.4)
Mycoplasma pneumoniae		
Positive rate in BALF, *n* (%)	71 (36.6)	202 (68.7)
Positive rate in NPA, *n* (%)	62 (31.9)	184 (62.5)
P	0.18	0.02
Sensitivity, % (95% *CI*)	67.6 (56.0–77.3)	81.2 (75.2–85.9)
Specificity, % (95% *CI*)	88.6 (81.8–93.1)	78.3 (68.8–85.4)
Positive predictive value, % (95% *CI*)	77.4 (65.6–86.0)	89.1 (83.8–92.8)
Negative predictive value, % (95% *CI*)	82.5 (75.2–88.1)	65.4 (56.1–73.6)
Cohen's kappa (95% *CI*)	0.6 (0.4−0.7)	0.6 (0.4−0.7)
Parainfluenza virus types 3		
Positive rate in BALF, *n* (%)	9 (4.6)	2 (0.7)
Positive rate in NPA, *n* (%)	20 (10.3)	3 (1.0)
P	0.03	
Sensitivity, % (95% *CI*)	33.3 (12.0–64.5)	/
Specificity, % (95% *CI*)	90.8 (85.7–94.1)	/
Positive predictive value, % (95% *CI*)	15 (5.2–36.0)	/
Negative predictive value, % (95% *CI*)	96.5 (92.6–98.4)	/
Cohen's kappa (95% *CI*)	0.1 (0.0–0.3)	/
Adenovirus		
Positive rate in BALF, *n* (%)	8 (4.1)	3 (1.0)
Positive rate in NPA, *n* (%)	9 (4.6)	4 (1.4)
P	1.00	1.00
Sensitivity, % (95% *CI*)	62.5 (30.5–86.3)	33.3 (6.1–79.2)
Specificity, % (95% *CI*)	97.8 (94.6–99.2)	98.9 (97.0–99.6)
Positive predictive value, % (95% *CI*)	55.5 (26.7–81.1)	25 (4.5–69.9)
Negative predictive value, % (95% *CI*)	98.4 (95.3–99.4)	99.3 (97.5–99.8)
Cohen's kappa (95% *CI*)	0.5 (0.4−0.7)	0.3 (0.1−0.4)

BALFs: Bronchoalveolar lavage fluids; NPA: Nasopharyngeal aspirate; *CI*: Confidence interval; /: Not calculated because of low positve rate.

golden standard for diagnose of LRTIs.[7-10] In this study, we found that the positive rate of pathogen detection in low respiratory tract was 61.6%, which was similar to the literature reported.[11,12] The mixed infection was 3.1%, which was lower than the results reported before.[13] Sp was still the most common bacterial pathogen.[14] Mp infection was very prevalent both in preschool children and old children.[15,16] PIV III and adenovirus were the main viruses detected in this study, which was different with reports before that most frequently detected viruses were RSV and rhinovirus,[17,18] and this might be related to the small proportion of infants <12 months (13.7%) in this study.

Due to the invasive nature of bronchoscopy, data on lower airway pathogens are very limited. Simultaneous NPA and BALF specimens have been reported even less. Here, we chose to perform this combined analysis to provide a large sample to evaluate the diagnostic accuracy of NPA in detecting pathogen in lower respiratory tract.

For bacterium, only the SP values were analyzed for the purpose of this study, due to the insignificant values obtained for the prevalence of other remaining bacterial infections. For Sp, the positive predictive value was very slight (20.8% and 20%) and the sensitivity decreased with ages. However, the specificity (89.9% and 97.2%) and negative predictive values (100% and 98.9%) of NPA cultures were high. Thus, a negative NPA culture indicated that Sp was unlikely to be present in the lower airways, while a positive NPA culture did not reliably predict the presence of lower airway organisms. Prior studies pertaining to the diagnostic accuracy of NPA culture focused predominantly on chronic pulmonary infection or cystic fibrosis,[19-21] therefore revealing an insufficiency in available data on acute LRTIs. Previous studies, not only sensitivities of NPA culture were very different (from 44% to 89%), but also the positive predictive values were very different (from 44% to 91%). These inconsistencies might be associated with the prevalence of bacterial infection. However, the specificity and negative predictive value narrowed within a limited range in these studies.[19-21] Therefore, we concluded that a negative NPA culture helped to "rule out" lower airway infection, while a positive NPA culture did not reliably "rule in" lower airway infection.

For Mp, there were acceptable diagnosis values in the sensitivity, specificity, positive predictive value, and negative predictive value in both age ranges. Moreover, the agreement of Mp detection results between NPA and BALF specimens was substantial (Cohen's kappa = 0.60). These results indicated that detection of Mp in NPA specimens has a fair discriminative power for predicting Mp infection in the lower respiratory tract. This should be related to high prevalence of Mp infection. Results obtained from this study revealed a positive correlation for Mp in BAL with a value of 55.9%, contributed predominantly by the high prevalence of Mp infection.[15-16] The method used for Mp detection was PCR, which was more sensitive than other traditional culture techniques,[22] and might also have contributed to the high positive rate that was acquired. However, this method might also account for partially false positive rates being observed due to the possibility that an exceedingly sensitive method might over amplify the results of BALF specimens that might be contaminated by Mp colonized in the nasopharynx.

For viruses, both PIV III and adenovirus revealed high values of specificity and a negative predictive value, while a slight sensitivity and a positive predictive value in both age ranges. Therefore, a negative NPA result indicated that isolation of PIV III or adenovirus from the lower airway was unlikely, while a positive NPA result did not reliably predict lower airway PIV III or adenovirus infection. The agreement of PIV III was very poor; however, the agreement of adenovirus was fair. This indicated that the adenovirus detected in NPA had some discriminative power for predicting adenovirus infection in lower respiratory tract. The low detection rate of viruses might be related to the detection method of DIA, which is less sensitive than PCR.[23]

There were limitations within this study. First, the children' clinical characteristics were not utilized, which might have assisted in distinguishing between colonization and infection. Second, fungal cultures were not performed and fungi are important pathogens of LRTIs. Third, for virus detection, PCR, which is more sensitive, was not implemented.

In conclusion, for bacterium and viruses, NPA specimens in children with LRTIs possess a high specificity and negative predictive value for lower airway pathogen but maintain poor sensitivity and a positive predictive value. For Mp, NPA specimen illustrated a good diagnostic value for Mp infection in the lower airway. Thus, a negative NPA result is helpful in "ruling out" lower airway infection, however, a positive result does not reliably "rule in" the presence of pathogen in the lower respiratory tract. These findings might have some implications for clinical management of children with LRTIs.

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Conflicts of interest

There are no conflicts of interest.

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