Bronchoscopic and Nonbronchoscopic Methods of Airway Culturing in Tracheostomized Children

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INTRODUCTION: Distal airway secretions can be sampled by bronchoscopic bronchoalveolar lavage (B-BAL), blind protected BAL (BP-BAL), and tracheal aspiration (TA). We quantitatively compared the cultures of distal airway secretions from BP-BAL, B-BAL, and TA specimens, and assessed the efficacy of the three above methods in diagnosing bronchitis in tracheostomized children. METHODS: Twenty children with tracheostomies underwent BP-BAL, B-BAL, and TA. Samples were sent for quantitative bacterial cultures. The diagnosis of bronchitis was made based on a validated visual grading system as well as on positive quantitative cultures from the BAL fluid. Diagnostic agreement between cultures obtained by the three methods and the visual grading scores was determined by kappa statistics. RESULTS: The diagnosis of bronchitis by visual grading score had substantial agreement with BP-BAL, moderate agreement with B-BAL, and fair agreement with TA results. BP-BAL specimens had significantly lower pathogenic colonies ($P < .05$) than either B-BAL or TA specimens. CONCLUSIONS: BP-BAL allows for more accurate sampling of lower airway secretions in tracheostomized children and is more accurate in the diagnosis of bronchitis in this group. *Key words: bronchoscopy; bronchoalveolar lavage; tracheostomy; aspirate; bronchitis; blind protected bronchoalveolar lavage.* [Respir Care 2014;59(4):582–587. © 2014 Daedalus Enterprises]

Introduction

Children with tracheostomies are susceptible to bacterial colonization and subsequent infections.[1-3] Bacterial cultures of tracheal secretions identify tracheal flora and facilitate the selection of antimicrobial therapy when clinically indicated. Respiratory tract samples are obtained using bronchoscopic as well as nonbronchoscopic techniques. Bronchoscopic bronchoalveolar lavage (B-BAL) bacterial cultures have a widely accepted diagnostic accuracy in the evaluation of patients with a variety of respiratory disorders.[4,5] B-BAL culture results may, however, be affected by contamination of the inner channel of the bronchoscope from upper airway secretions.[6] The blind protected BAL (BP-BAL) method has the potential of obtaining noncontaminated samples of lower respiratory tract secretions.[7] Kollef et al[7] demonstrated that BP-BAL can be performed safely on mechanically ventilated children in the pediatric ICU, whereas Fujitani et al[8] showed the limitations of cultures of endotracheal aspirate in the diagnosis of ventilator-associated pneumonia (VAP) compared to cultures of the lower respiratory tract using non-B-BAL in similar patients. However, these studies were performed in intubated patients with nosocomial pneumonia. To date, there are no studies on the clinical efficacy of nonbronchoscopic techniques of BAL in pediatric tracheostomized patients. We hypothesized that BP-BAL is as effective as B-BAL and more accurate than tracheal aspiration (TA) in the sampling of lower airway secretions in children with long-term tracheostomies.

We compared three different methods of obtaining distal airway cultures, which include B-BAL, BP-BAL, as well as TA, in children with tracheostomies.

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The authors have disclosed no conflicts of interest.

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Methods

Objective and Design

Through an observational study of 20 subjects with long-term tracheostomies, we compared three methods of obtaining distal airway cultures, namely, B-BAL, BP-BAL, and TA. The patients were seen in the outpatient clinic in the Division of Pediatric Pulmonology at Maimonides Infants and Children's Hospital of Brooklyn, and caregivers provided informed consent prior to study enrollment. The study was approved by the Maimonides Medical Center Institutional Review Board.

QUICK LOOK

Current knowledge

Distal airway secretions can be sampled by bronchoscopic bronchoalveolar lavage, blind protected bronchoalveolar lavage and tracheal aspirates. Eash method has advantages and disadvantages.

What this paper contributes to our knowledge

Blind protected bronchoalveolar lavage allowed for more accurate sampling of lower airway secrections in tracheostomized children and was more accurate in the diagnosis of bronchitis in this group compared to the other methods.

Patients

The study was performed in the pediatric bronchoscopy suite at Maimonides Infants and Children's Hospital of Brooklyn between October 2010 and December 2010. Children with tracheostomies for at least 6 months and who were undergoing either elective tracheostomy evaluation (surveillance bronchoscopy) or airway endoscopy due to clinical evidence of respiratory infection such as a change in color or an increase in the volume of secretions were included. We excluded patients with evidence of cardiopulmonary instability, which could jeopardize their clinical status during the procedure.

Collecting Lower Airway Secretions

Each patient was ventilated with 100% oxygen and sedated with intravenous propofol (1 mg/kg intravenously followed by 0.5 mg/kg every 3 to 5 min as needed for sedation). Blood pressure, heart rate, and oxygen saturation were monitored during the entire procedure. BP-BAL was performed using a plugged telescoping catheter (Fig. 1) as per the manufacturer's instructions[9] (Combicath, KOL Bio-Medical Instruments, Chantilly, Virginia).

Fig. 1. Combicath catheter.

The sequence of collecting the specimens was as follows: BP-BAL followed by B-BAL, and finally TA.

In the BP-BAL technique, the telescoping catheter (Combicath) was advanced through the tracheostomy tube until resistance was met, indicating that the catheter was wedged in the distal airway. The catheter was then pulled out about 3 cm to allow room for the inner catheter to be advanced. The white plastic protective spacer that separates the inner and outer catheter was then removed. We gently advanced the inner catheter to connect it to the outer catheter to dislodge the absorbable polyethylene glycol plug at the catheter tip. The catheter was flushed with one to two aliquots of 1 mL/kg normal saline solution and aspirated. The aspirate was collected and transferred into a sterile specimen trap (Tyco Healthcare Group, Mansfield, Massachusetts). A minimum of 5 mL of aspirate was considered sufficient for analysis.

B-BAL was performed using a flexible fiberoptic bronchoscope (Olympus America, Center Valley, Pennsylvania). Macroscopic assessments of the presence of lower airway inflammation and quantification of lower airway secretions were performed. BAL fluid was obtained from the lobe that appeared to have the most secretions. If the secretions were seen throughout the lower airways, the BAL bronchoscope was wedged in the right middle lobe. The bronchial segment that had evidence of irritation from the telescoping catheter (Combicath) was avoided to decrease the chance of specimen contamination. The bronchoscope was wedged in a desired subsegmental bronchus, flushed with 2 to 3 aliquots of 1 mL/kg normal saline solution and suctioned with a pressure of 50–80 mm Hg into a sterile specimen trap until a minimum of 5 mL of aspirate was obtained.[10]

Last, TA was performed with an aspiration catheter (CareFusion, San Diego, California), which was inserted

Table 1. Duration of Procedure and Number of Bacterial Colonies in the Cultures of Distal Airway Secretions Collected by the 3 Techniques

Variables	BP-BAL	B-BAL	TA	*P* value
Duration of procedure, min	3.0 (2.0, 3.8)	7.0 (6.3, 8.0)	2.0 (1.5, 2.0)	< .05
Total pathogenic colonies, CFU/mL	150 (0, 16,050)	32,500 (8,100, 57,600)	40,500 (18,000, 78,550)	< .05
Total nonpathogenic colonies, CFU/mL	1,500 (0, 3,000)	20,250 (7,500, 40,000)	32,500 (10,000, 60,000)	< .05

All values are given as the median (25th, 75th percentiles).
BP-BAL = blind protected bronchoalveolar lavage
B-BAL = bronchoscopic bronchoalveolar lavage
TA = tracheal aspiration
CFU = colony-forming units

through the tracheostomy tube until resistance was met and was flushed with 2 or 3 aliquots of 1 mL/kg normal saline solution, and fluid was aspirated into a sterile specimen trap. A minimum of 5 mL of aspirate was considered sufficient for analysis.

The samples obtained from each procedure were sent to the microbiology laboratory immediately for quantitative culture and analysis.

Quantification of Lower Airway Secretions

During B-BAL the tracheobronchial tree was visually assessed to quantify secretions. The secretions were quantified according to a bronchoscopy secretion scoring system—a visual grading score (VGS) that has been described elsewhere.[11,12] Secretions were graded on a scale from 1 to 6 based on the severity of the secretions. Subjects with secretion grades 1 and 2 were considered to have no bronchitis; those with grade 3 had minimal bronchitis; and those with grades 4–6 had mild, moderate, and severe bronchitis, respectively.[11]

Microbiological Analysis

Tubes containing the specimens were vortexed for 10–20 s. A 0.01 calibrated loop was inoculated and plated onto blood agar, chocolate agar, and MacConkey agar Petri dishes (BD, Franklin Lakes, New Jersey) using the standard technique. Plates were then incubated in 5–7% CO_2 at 35–37°C for 48 h. Growth was quantitated according to colony-forming units (CFU)/mL on each plate. All microorganisms and their susceptibilities were reported. No growth indicated no organisms isolated. All growth $\geq 10^2$ CFU/mL was reported. Positive thresholds for the sampling methods were defined as $\geq 10^2$ CFU/mL for BP-BAL samples,[9] and $\geq 10^4$ CFU/mL for B-BAL and TA samples.[8]

Statistical Analysis

All statistical analysis was performed using statistical software (SPSS 19, SPSS, Chicago, Illinois). The continuous numerical values are expressed as the mean ± SD or as the median (25th, 75th percentiles). The significance of differences in values among B-BAL, BP-BAL, and TA procedures was evaluated using the Kruskal-Wallis one-way analysis of variance on ranks. Post hoc all pairwise multiple comparisons were performed using the Tukey test. The percentage of concordance was calculated as the ratio of total agreements in the diagnosis of bronchitis by each culture technique versus the total number of cultures (20%). The agreements between the VGS and the 3 techniques of distal airway secretion cultures, or among the 3 techniques of distal airway secretion cultures in the diagnosis of bronchitis, were statistically evaluated by calculating the Cohen's kappa coefficient.

Results

Of the 20 patients enrolled in the study 13 were males (65%) and 7 were females (35%). The mean (±SD) age of the patients was 12.7 ± 8.9 years. The duration of tracheostomy was 6.2 ± 6.7 years. The median duration for each procedure was 3.0 min (1.2–5.0 min) for BP-BAL, 7.0 min (5.0–15 min) for B-BAL, and 2.0 min (1.0–3.0 min) for TA. The BP-BAL procedure was significantly shorter ($P < .05$) than the B-BAL procedure (Table 1).

A total of 30 different bacterial strains were isolated from study patients. Seventeen microorganisms were deemed pathogenic (Table 2), and 13 microorganisms were nonpathogenic (Table 2). The most frequently isolated pathogenic organism, irrespective of technique, was *Pseudomonas aeruginosa* (Table 2).

The median number of pathogenic colonies isolated was 150 CFU/mL in BP-BAL samples, 32,500 CFU/mL in B-BAL samples, and 40,500 CFU/mL in TA samples (Table 1). BP-BAL samples had significantly fewer pathogenic colonies ($P < .05$) than either B-BAL or TA samples. The median number of nonpathogenic colonies was 1,500 CFU/mL in BP-BAL samples, 20,250 CFU/mL in B-BAL samples, and 32,500 CFU/mL in TA samples. BP-BAL samples had significantly fewer nonpathogenic colonies ($P < .05$) compared to either B-BAL or TA samples (Table 1).

Table 2. Pathogenic and Nonpathogenic Bacterial Species Identified in the Cultures of Distal Airway Secretions Collected by the 3 Techniques

Bacterial Species	BP-BAL	B-BAL	TA
Pathogenic			
No growth	4	0	0
Acinetobacter baumannii	1	1	1
Acinetobacter haemolyticus	1	1	1
Alpha-hemolytic *Streptococcus*	4	4	4
Beta-hemolytic *Streptococcus*	3	3	3
Citrobacter koseri	0	1	1
Escherichia coli	0	1	2
Haemophilus influenzae	2	1	1
Haemophilus parainfluenzae	0	2	2
Klebsiella oxytoca	1	1	1
Klebsiella pneumonia	0	1	1
MRSA	1	2	2
MSSA	3	4	3
Proteus mirabilis	0	1	0
P. aeruginosa strain 1	6	10	10
P. aeruginosa strain 2	0	1	1
Serratia marcescens	3	5	4
Streptococcus pneumonia	3	4	4
Nonpathogenic			
No growth	4	0	0
Achromobacter denitrificans	1	1	1
Corynebacterium jeikeium	0	1	1
Corynebacterium minutissimum	1	1	1
Corynebacterium pseudo striatum	1	0	0
Corynebacterium species	3	4	4
Corynebacterium striatum	4	8	8
Morganella morganii	0	1	1
Neiserria cinerea	1	2	2
Neiserria elongata	1	1	1
Neiserria sicca strain 1	2	3	3
Neiserria sicca strain 2	1	1	1
Normal respiratory flora	1	3	4
Staphylococcus epidermidis	0	1	1

Values represent the number of patients with the specific bacterial strain.
BP-BAL = blind protected bronchoalveolar lavage
B-BAL = bronchoscopic bronchoalveolar lavage
TA = tracheal aspiration
MRSA = methicillin-resistant *Staphylococcus aureus*
MSSA = methicillin-sensitive *Staphylococcus aureus*

Diagnosis of bronchitis by VGS had substantial agreement (kappa = 0.7; concordance, 85%) with BP-BAL quantitative cultures, but only moderate agreement (kappa = 0.5; concordance, 75%) with B-BAL cultures, and fair agreement (kappa = 0.3; concordance, 65%) with TA cultures (Table 3). Among the three techniques, the diagnostic agreement was substantial between BP-BAL and B-BAL cultures (kappa = 0.76; concordance, 90%), but only moderate between BP-BAL and TA cultures (kappa = 0.49; concordance, 80%) (Table 4).

Table 3. Agreement Between the VGS and the 3 Techniques of Distal Airway Secretion Cultures in the Diagnosis of Bronchitis

Technique	Concordance, %	Kappa Value	Strength of Agreement
BP-BAL vs VGS	85	0.70	Substantial
B-BAL vs VGS	75	0.50	Moderate
TA vs VGS	65	0.30	Fair

BP-BAL = blind protected bronchoalveolar lavage
B-BAL = bronchoscopic bronchoalveolar lavage
TA = tracheal aspiration
VGS = visual grading score

Table 4. Agreement Among the 3 Techniques of Distal Airway Secretion Cultures in the Diagnosis of Bronchitis.

Technique	Concordance, %	Kappa Value	Strength of Agreement
TA vs B-BAL	90	0.69	Substantial
TA vs BP-BAL	80	0.49	Moderate
BP-BAL vs B-BAL	90	0.76	Substantial

BP-BAL = blind protected bronchoalveolar lavage
B-BAL = bronchoscopic bronchoalveolar lavage
TA = tracheal aspiration

Visualization of the side of the bronchi in which the telescoping catheter (Combicath) was wedged during B-BAL was performed in 7 subjects (35%). This was done by introducing the flexible bronchoscope alongside the wedged catheter. Of these subjects, 5 (71%) had the catheter wedged in the right bronchus, while 2 subjects (29%) had it wedged in the left bronchus.

Discussion

Our study showed substantial agreement between the diagnosis of bronchitis based on VGS and quantitative cultures obtained by BP-BAL, moderate agreement with cultures obtained by B-BAL, and only fair agreement with cultures obtained by TA. Bronchoscopic diagnosis of bronchitis is based on visual assessment and can be confirmed by the analysis of BAL cellularity. The bronchitis index, first introduced by Thompson et al,[12] was based on visual assessment of inflammatory changes in the bronchi correlated with neutrophil percentage in BAL fluid samples. Later, Chang et al[11] introduced and validated a scoring system for bronchoscopic secretions. They studied a cohort of 106 children, quantified the amount of secretions present in the airways of those children during bronchoscopy, and showed that the secretions score correlated with airway cellularity, associated airway neutrophilia, as well as with the airways' infective state. Similarly, we used a

VGS of 4 as a "cutoff value" for the diagnosis of bronchitis.

We demonstrated a substantial agreement between the results of quantitative cultures using BP-BAL and B-BAL, but only moderate agreement between the results using BP-BAL and TA. Data obtained in children with VAP suggested that the blind protected technique of sampling lower airway secretions is similar in efficacy to the bronchoscopic method[8] and is superior to endotracheal aspiration.[13]

The respiratory tracts of patients with tracheostomies are colonized soon after placement.[1] Brook[1] showed that 100% of children with tracheostomies are colonized with bacteria as early as 2 weeks after placement of tracheostomies. These patients frequently present with clinically apparent airway infections noted by increased secretions, increased oxygen requirements, and/or a new infiltrate seen on a chest x-ray.[3,14]

The endoscopic assessment of children with tracheostomies during elective bronchoscopy has multiple purposes. These purposes include evaluation of the airways, detection of underlying airway pathology, assessment of tracheostomy tube sizing and positioning, and determination of readiness for decannulation. The recent study by Cline et al[15] questions the reliability of surveillance cultures in children with tracheostomies due to the poor reproducibility of the cultures. However, in the cases of clinically apparent airway infections, cultures and susceptibilities should be obtained to guide therapy. For example, *P. aeruginosa* was the most common pathogenic organism isolated in our cohort of patients. This finding is in agreement with previously published data[8,16] and highlights the importance of culturing lower respiratory tract secretions to make the appropriate antibiotic choice for treatment.

The reliability of the lower airway culture and its accuracy remain important issues. Sachdev et al[16] reported that endotracheal aspirate cultures have high false-positive rates in microbiological diagnostics of VAP in children secondary to colonization of proximal airways. The suction channels of flexible bronchoscopes are frequently contaminated with upper airway flora during instrument insertion.[6,13]

The concept of "quantitative cultures" was introduced by several investigators[5,8,17] to improve the sensitivity and specificity of microbiological diagnostics. The thresholds for positive cultures differ depending on patient population and the technique used to obtain the culture. For example, cystic fibrosis practitioners tend to use higher thresholds for BAL quantitative cultures (usually 10^5 CFU/mL),[4,18] whereas the threshold of 10^4 CFU/mL is considered to be acceptable for the diagnosis of lower respiratory tract infections in non-cystic fibrosis patients.[5] Different diagnostic thresholds for positive quantitative cultures have been established for BP-BAL, B-BAL, and TA by previous investigators[7,16,19] and were used in our study. The lowest threshold of 10^2 for BP-BAL as applied in our study was also used by Timsit et al,[20] and it provided a higher sensitivity in determining significant positivity of the cultures obtained, although specificity was somewhat decreased.

In our study BP-BAL samples showed significantly fewer colonies for both pathogenic and nonpathogenic organisms than samples obtained with both B-BAL and TA. This may suggest that samples obtained with BP- BAL are less contaminated with upper airway flora and thus identify causative organisms of lower airway infection in children with tracheostomies more accurately.

BP-BAL was performed in significantly less time than B-BAL, was technically straightforward, and did not require special training. However, BP-BAL may have potential disadvantages when compared with B-BAL. Lack of visual control when performing BP-BAL makes the site of aspiration "random," precluding the precise sampling of bronchial segments most affected by an infectious process. For example, BP-BAL cultures were negative in the presence of positive B-BAL and TA cultures in 4 patients in our study. Of note, 2 of these patients had bronchitis based on the VGS, thereby questioning the specificity of cultures as a result of the lack of visual guidance. This limitation could be eliminated by passing the protected catheter via the bronchoscope channel. Unfortunately, the presently available sizes of protected catheters and pediatric bronchoscopy channels limit the utility of this technique.

No major adverse events from any of the three diagnostic procedures were observed in this study. Minor or moderate bronchial hemorrhage, pneumothorax, and an increase in oxygen or ventilatory requirements, especially in children with poor oxygenation indices, have been previously reported in children after they had undergone BP-BAL.[21]

Our study has several limitations. The small size of the tracheostomy tube did not allow us to introduce the flexible bronchoscope alongside the blind protected catheter to detect the BP-BAL sampling sites in all patients. Consequently, we were not able to precisely determine whether the difference in sampling sites between BP-BAL and BAL affected the culture results. Our group of patients was fairly heterogeneous, and the results could not be adjusted for several factors, such as duration of tracheostomy, time interval since the last tracheostomy tube change, and severity and frequency of lower respiratory tract infections. The relatively small number of patients enrolled in the study did not provide a sample size sufficient to resolve the above-mentioned problems. Finally, our results, although favoring the use of the BP-BAL method in children with tracheostomies, cannot be extrapolated to other patient populations.

In conclusion, BP-BAL is a reliable method of obtaining lower respiratory tract secretions for culturing in children with tracheostomies. It is relatively easy, quick, and

safe, and can be performed by residents, respiratory therapists, and nurses. Further research studies are indicated to fully reach the potential of the BP-BAL method in other patients.

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