

Comparison of Bronchoscopic and Nonbronchoscopic Methods of Airway Culturing in Tracheostomized Children.

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Abstract

Introduction:

Distal airway secretions can be sampled by bronchoscopic bronchoalveolar lavage (B-BAL), blind protected BAL (BP-BAL) and tracheal aspirates (TA). We quantitatively compared the cultures of distal airway secretions from BP-BAL, B-BAL, and TA and assessed the efficacy of the three above methods in diagnosing bronchitis in tracheostomized children.

Method:

Twenty children with tracheostomies underwent BP-BAL, B-BAL and TA. Samples were sent for quantitative bacterial cultures. Diagnosis of bronchitis was made based on a validated visual grading system as well as on positive quantitative culture in the BAL fluid. Diagnostic agreement between cultures obtained by the three methods and the visual grading scores was determined by kappa statistics.

Results:

Diagnosis of bronchitis by visual grading score had substantial agreement with BP-BAL, moderate agreement with B-BAL, and fair agreement with TA. BP-BAL had significantly lower pathogenic colonies ($P < 0.05$) than either B-BAL or TA.

Conclusion:

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.

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 evaluation of patients with 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 bronchoalveolar lavage method (BP-BAL) has the potential of obtaining non-contaminated samples of lower respiratory secretions (7). Kollef et al (7) demonstrated that BP-BAL can be performed safely on mechanically ventilated children in the pediatric intensive care unit while Fujitani et al (8) showed the limitations of cultures of endotracheal aspirate in the diagnosis of VAP compared to cultures of the lower respiratory tract using non-bronchoscopic BAL in similar patients. However these studies were done on intubated patients with nosocomial pneumonia. To date, there are no studies on the clinical efficacy of non-bronchoscopic techniques of BAL in pediatric tracheostomized patients. We hypothesized that BP-BAL is as effective as B-BAL and more accurate than TA in sampling of lower airway secretions in children with long term tracheostomies.

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

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 followed by the division of Pediatric Pulmonology at Maimonides Infants and Children's Hospital of Brooklyn and care givers provided informed consent prior to enrollment. The study was approved by the Maimonides Medical Center Institutional Review Board.

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 change in color or increase in 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 IV 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 as per manufacturer's instructions (9) (Combicath®, Kol BioMedical instruments Inc, VA).

The sequence of collecting the specimens was: BP-BAL followed by B-BAL and finally tracheal aspiration.

In the BP-BAL technique, the 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 3cm to allow room for the inner catheter to be advanced. The white plastic protective spacer which separates the inner and outer catheter was then removed. We gently advance the inner catheter to connect it to the outer catheter in order to dislodge the absorbable polyethylene glycol plug at the catheter tip. The catheter was flushed with one to two aliquots of 1ml/kg of normal saline and aspirated. The aspirate was collected and transferred into a sterile specimen trap (Tyco Health Care Group, MA). A minimum of 5mls of aspirate was considered sufficient for analysis.

B-BAL was performed using a flexible fiberoptic bronchoscope (Olympus America Inc.) Macroscopic assessments of the presence of lower airway inflammation and quantification of lower airway secretions were performed. BAL was obtained from the lobe that appeared to have the most secretions. If the

secretions appeared diffuse, the BAL was wedged from the right middle lobe. The bronchial segment that had evidence of irritation from the combicath was avoided to decrease the chance of specimen contamination. The bronchoscope was wedged in a desired sub segmental bronchus, flushed with 2 to 3 aliquots of 1ml/kg of normal saline and suctioned with a pressure of 50- 80mmHg into a sterile specimen trap until a minimum of 5mls of aspirate was obtained(10). Lastly, TA was performed with an aspiration catheter (Carefusion, CA), which was inserted through the tracheostomy tube until resistance was met, flushed with 2 or 3 aliquots of 1ml/kg normal saline and aspirated into a sterile specimen trap. A minimum of 5 mls 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 –visual grading score (VGS) described elsewhere (11, 12). Secretions were graded from 1 to 6 based on severity of secretions. Subjects with secretion grade 1 and 2 were considered to have no bronchitis, grade 3 had minimal and those graded 4 to 6 had mild, moderate and severe bronchitis respectively.(11)

Microbiological analysis

Tubes containing the specimens were vortexed for 10-20 seconds. A 0.01 calibrated loop was inoculated and plated onto blood agar, chocolate agar and MacConkey agar Petri dishes(BD, NJ) using standard technique. Plates were then incubated in 5 to 7% CO₂ at 35 to 37 degrees Centigrade for 48 hours. Growth was quantitated according to colony forming units per milliliter (cfu/ml) on each plate. All microorganisms and their susceptibilities were reported. No growth indicated no organisms isolated. All growth greater than or equal to (\geq) 10^2 cfu/ml was reported. Positive thresholds for the sampling methods were defined as $\geq 10^2$ cfu/ml for BP-BAL(9) and $\geq 10^4$ cfu/ml for B-BAL and TA samples.(8)

Statistical Analysis

All statistical analysis was performed using SPSS (version 19) software. The continuous numerical values are expressed as Mean \pm S.D. or as Median (25th, 75th percentiles. Significance of differences in values between B-BAL, BP-BAL, and TA procedures were evaluated using Kruskal-Wallis One Way Analysis of Variance on Ranks. Post-hoc all pairwise multiple comparisons were performed by using Tukey Test. Percent concordance was calculated as the ratio of total agreements in 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 (\pm 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 in minutes was 3.0 (1.2 to 5.0) for BP-BAL, 7.0 (5.0 to 15) for B-BAL and 2.0 for TA (1.0 to 3.0). BP-BAL procedure was significantly shorter ($p<0.05$) than the B-BAL procedure (Table 1).

A total of 29 different bacterial strains were isolated from study patients. Fifteen microorganisms were deemed pathogenic (Table 2A) and 14 microorganisms were non-pathogenic (Table 2B). The most frequently isolated pathogenic organism irrespective of technique was *Pseudomonas aeruginosa* (Table 2A).

The median number of pathogenic colonies isolated was 150 cfu/ml in BP-BAL, 32500 cfu/ml in B-BAL, and 40500 TA (Table 1). BP-BAL had significantly fewer pathogenic colonies ($P<0.05$) than either B-BAL or TA. The median number of nonpathogenic colonies were 1500 cfu/ml in BP-BAL, 20250 cfu/ml in B-BAL and 32500 cfu/ml in TA. BP-BAL had significantly fewer nonpathogenic colonies ($P<0.05$) also compared to either B-BAL or TA (Table 1).

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, and fair agreement

(Kappa 0.3, concordance 65%) with TA (Table 3). Among the three techniques, the diagnostic agreement was substantial between BP-BAL and B-BAL (Kappa 0.76, concordance 90%), but only moderate between BP-BAL and TA (Kappa 0.49, concordance 80%) (Table 4).

Visualization of the side of the bronchi in which the 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, 5 (71%) had the catheter wedged in the right bronchus while 2 (29%) had it wedged in the left.

Discussion

Our study showed substantial agreement between the diagnosis of bronchitis based on VGS and quantitative cultures obtained by BP-BAL, moderate agreement with B-BAL and only fair agreement with 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. Later, Chang et al(11) introduced and validated a bronchoscopic secretions scoring system. They studied a cohort of 106 children, quantified the amount of secretions present in their airway during bronchoscopy and showed that the secretions score correlated with both airway cellularity, associated airway neutrophilia, as well as with the airway's infective state. Similarly, we used a VGS of 4 as a "cut-off value" for the diagnosis of bronchitis.

We showed a substantial agreement between the results of quantitative cultures between the BP-BAL and B-BAL while there was only moderate agreement between BP-BAL and TA. Data obtained in children with VAP suggested that blind protected technique of sampling lower airway secretions is similar in efficacy to bronchoscopic method (8) and superior to endotracheal aspiration(13) The respiratory tracts of patients with tracheostomies are colonized soon after placement (1). Brook et al (1) showed that 100% of children with tracheostomies are colonized with bacteria as early as two 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 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 their poor reproducibility. However, in the cases of clinically apparent airway infections, cultures and susceptibilities should be obtained to guide therapy. For example, *Pseudomonas 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 in order 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 rate 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) in order 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 (CF) practitioners tend to use higher thresholds for BAL quantitative cultures (usually 10^5 cfu/ml)(4, 18) while the threshold of 10^4 cfu/ml is considered to be acceptable for the diagnosis of lower respiratory tract infections in non-CF patients.(5) Different diagnostic thresholds for positive quantitative cultures are established for BP-BAL, B-BAL and TA by previous investigators(7, 16, 19) and were used in our study. Lowest threshold of 10^2 for BP-BAL as applied in our study was also used by Timsit et al and it provided a higher sensitivity in determining significant positivity of cultures obtained. Although specificity was somewhat decreased (20)

In our study BP-BAL showed significantly fewer colonies for both pathogenic and nonpathogenic organisms than 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 infectious process. For example, BP-BAL cultures were negative in the presence of positive B-BAL and TA in four patients in our study. Of note, two of these patients had bronchitis based on the VGS, thereby questioning the specificity of cultures due to lack of visual guidance. This limitation could be eliminated by passing the protected catheter via the bronchoscope channel. Unfortunately, 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 increase in oxygen or ventilatory requirement especially in children with poor oxygenation indices have been previously reported in children after BP-BAL.(21) Our study has several limitations. The small size of 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 if the difference in sampling sites between BP-BAL and BAL affected the cultures 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 infections. The relatively small number of patients enrolled in the study did not provide the sample size sufficient to resolve the above mentioned problems. Finally, our results, although favor the use of BP-BAL method in children with tracheostomies cannot be extrapolated to other patient populations.

In conclusion, BP-BAL is a reliable method of lower respiratory secretions culturing in children with tracheostomies. It is relatively easy, quick, safe and can be performed by residents, respiratory therapists and nurses. Further research studies are indicated to fully reach the potential of BP-BAL method in other patients.

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Table 1. Duration of procedure and number of bacterial colonies in the cultures of distal airway secretions collected by the 3 techniques

	B P - B A L	B - B A L	Tracheal Aspirate	P value
Duration of Procedure(mins)	3.0 (2.0,3.8)*	7.0 (6.3, 8.0)	2.0 (1.5, 2.0)	<0.05
Total Pathogenic Colonies (cfu/ml)	150 (0,16050)	32500 (8100, 57600)	40500(18000, 78550)	<0.05
Total Non- Pathogenic Colonies (cfu/ml)	1500 (0, 3000)	20250 (7500,40000)	32500 (10000,60000)	<0.05

*All values are given as Median (25th, 75th percentiles).

Table 2A. Pathogenic bacterial species identified in the cultures of distal airway secretions collected by the three techniques

Pathogenic bacterial species	BP-BAL	B-BAL	TA
No growth	4*	0	0
<i>Acinetobacter baumannii</i>	1	1	1
<i>Acinetobacter hemolyticus</i>	1	1	1
Alpha Hemolytic Strept	4	4	4
Beta Hemolytic Strept	3	3	3
<i>Citrobacter koseri</i>	0	1	1
<i>E.Coli</i>	0	1	2
<i>Hemophilus influenza</i>	2	1	1
<i>Hemophilus parainfluenza</i>	0	2	2
<i>Klebsiella oxytoca</i>	1	1	1
<i>Klebsiella pnemoniae</i>	0	1	1
MRSA	1	2	2
MSSA	3	4	3
<i>Proteus mirabilis</i>	0	1	0
<i>Pseudomonas aeruginosa</i> Strain 1	6	10	10
<i>Pseudomonas aeruginosa</i> Strain 2	0	1	1
<i>Serratia marcescens</i>	3	5	4
<i>Strept pneumoniae</i>	3	4	4

*Values represent the number of patients with the specific bacterial strain

Table 2B. Nonpathogenic bacterial species identified in the cultures of distal airway secretions collected by the three techniques

Nonpathogenic Bacterial Species	BP-BAL	B-BAL	TA
No growth	4	0	0
<i>Achromobacter denitrificans</i>	1	1	1
<i>Corynebacterium jeikeium</i>	0	1	1
<i>Corynebacterium minutissimum</i>	1	1	1
<i>Corynebacterium pseudostriatum</i>	1	0	0
<i>Corynebacterium</i> Sp	3	4	4
<i>Corynebacterium striatum</i>	4	8	8
<i>Morganella morganii</i>	0	1	1
<i>Neisseria cinerea</i>	1	2	2
<i>Neisseria elongata</i>	1	1	1
<i>Neisseria sicca</i> Strain 1	2	3	3
<i>Neisseria sicca</i> Strain 2	1	1	1
Normal Resp Flora	1	3	4
<i>Staph. epidermidis</i>	0	1	1

Values represent the number of patients with the specific bacterial strain

Table 3. Agreement between the visual grading score (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

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

