Nonbronchoscopic bronchoalveolar lavage for diagnosing ventilator-associated pneumonia in newborns

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The appropriate treatment of ventilator-associated pneumonia (VAP) must be based on accurate diagnosis, which can be done by microbiological examination of the samples obtained from the respiratory tract by nonbronchoscopic bronchoalveolar lavages (NB-BAL).

This study was designed to determine the effectiveness of NB-BAL in diagnosing VAP in newborns.

Two hundred and seven NB-BAL samples were obtained from 145 intubated neonates for microbiologic and cytologic evaluation of the distal airway. The NB-BAL samples were processed for microscopic quantification of the polymorphonuclear cells (PMN) containing intracellular bacteria (ICB) and quantitative culture (positive threshold, 10^5 cfu/ml). VAP was defined as a new, progressive, or persistent (>24 hrs) infiltrate on the chest radiograph, with two or more of the following criteria: a) macroscopically purulent tracheal secretions, b) fever or hypothermia, c) leukocytosis or leukopenia, and d) worsening of respiratory status with a Pa O_2/F IO_2 ratio of <240. Colonization was defined as mechanical ventilation for more than seven days, no signs of infection, and isolation of the same bacteria species in two previously obtained NB-BAL samples.

Of the 145 neonates, 40 (27.5%) were infected and 12 (8.3%) were colonized. Forty-four patients (30%) developed VAP according to diagnostic categories based on clinical and radiologic criteria. Forty newborns with VAP (90%) had positive NB-BAL culture. The sensitivity, specificity, and positive and negative predictive values of NB-BAL fluid culture for VAP diagnosis were 90%, 90%, 70%, and 97%, respectively. The percentage of ICB was significantly higher in newborns with VAP. The presence of ICB in 2% or more on Giemsa-stained smears corresponded to a sensitivity of 94%, specificity of 83%, positive predictive value of 94%, and negative predictive value of 83%. The sensitivity and specificity of combination of ICB and NB-BAL quantitative culture in diagnostic samples were 94% and 90%, respectively. The positive and negative predictive values were 71% and 98%.

In our study, the presence of leukocytes in the NB-BAL fluid smear of infants with VAP was higher than that of the colonized babies (84%, 26%). This difference was statistically significant ($p<0.0001$). The sensitivity and specificity of PMNs in NB-BAL fluid for the diagnosis were 86% and 75%, respectively, and the positive and negative predictive values were 89% and 69%.

We conclude that NB-BAL lavage is well tolerated and clinically useful in mechanically ventilated newborns. These results suggest that NB-BAL fluid microscopic examination and cultures can offer a sensitive and specific means to diagnose VAP in newborns and may provide relevant information about the causative pathogens.

Key words: nonbronchoscopic bronchoalveolar lavage, ventilator-associated pneumonia, newborn, sepsis, mechanical ventilation, premature.

Ventilator-associated pneumonia (VAP) is a common and severe complication of mechanical ventilation[1]. Its rate of occurrence ranges from 10% to 70% depending on the study population and diagnostic criteria. Substantial efforts have been devoted to improving the means for early and accurate diagnosis of VAP in neonatal intensive care unit (NICU) patients because of its high incidence and mortality[2]. The diagnosis of pneumonia in intubated, ventilated patients is difficult to confirm. This relates to the lack of specificity of physical examination findings, diagnostic imaging, cultures, and other laboratory tests. Organisms recovered from the upper airway or by suction through an endotracheal tube may be colonizers and not represent lower respiratory flora. Even bronchoscopic specimens may be contaminated by pharyngeal flora. Advances have been made in diagnosing VAP by quantitating the number of organisms recovered in bronchoalveolar lavage fluid. Organisms recovered in large numbers are the presumed etiologic agent[3]. Bronchoscopic bronchoalveolar lavage (B-BAL) has become a routine procedure for obtaining a sample of bronchoalveolar lavage fluid (BALF) in both adult and pediatric populations, including children requiring mechanical ventilation due to severe lung disease. B-BAL has been evaluated extensively, using quantitative culture techniques and microscopic identification of intracellular bacteria (ICB) in cells retrieved by B-BAL[4]. However, B-BAL is associated with deterioration of pulmonary mechanics and function as well as hypotension and pyrexia[5]. Alternatives include tracheal aspirate (TA) and non-bronchoscopic bronchoalveolar lavage (NB-BAL). Both are minimally invasive and relatively inexpensive techniques[6]. Several studies have shown that diagnostic accuracy of NB-BAL is similar to that of B-BAL[7], and NB-BAL has therefore been used as a simple and inexpensive alternative to B-BAL[7,8]. The aim of this prospective study was to determine the effectiveness of NB-BAL fluid microscopic examination and culture in diagnosing VAP in newborns.

Material and Methods

This study was performed between January 1998 and May 2001 at the NICU of our hospital, with the approval of the ethical committee. Informed consent was obtained from the parents.

Non-bronchoscopic bronchoalveolar lavage (NB-BAL) was carried out in 145 neonates who were intubated and ventilated between January 1998 and May 2001 by intermittent mandatory ventilation. All consecutive patients who received mechanical ventilation for >48 hours were included. The bacteriology of NB-BAL from 145 newborns was studied. Specimens were obtained twice a week as long as the newborns were intubated. Clinical suspicion of VAP was defined as a new, progressive, or persistent (>48 hrs) infiltrate on the chest radiograph, with two or more of the following criteria: a) macroscopically purulent tracheal secretions, b) temperature of ≥38.5°C or <36.5°C, c) leukocytosis of ≥20,000 cells/mm^3 or leukopenia of <4,000 cells/mm^3, and d) worsening of respiratory status with a Pa O_2/FiO_2 ratio of <240. Colonization was defined as mechanical ventilation for more than seven days, no signs of infection and isolation of the same bacteria species in two consecutive samples of NB-BAL samples (9). All babies were followed for clinical findings (poor feeding, lethargy, apnea, tachypnea, jaundice, and temperature of ≥38.5°C or <36.5°C) and laboratory findings of neonatal sepsis (leukocytosis of ≥20,000 cells/mm^3 or leukopenia <5,000 cells/mm^3, thrombocytopenia, toxic granulation, vacuolization, total nonsegmented neutrophil count, immature/mature ratio), and were evaluated for pulmonary infection. The criteria for diagnosis of sepsis were as follows: presence of positive blood culture, or negative blood culture with the newborn presenting clinical signs of infection[10].

Sampling Procedure

The patients were all intubated, ventilated, and monitored by pulse oximeter, electrocardiogram, and intra-arterial blood pressure measurement. During the procedure, ventilation was provided by a T-Ayres bagging circuit delivering an FiO_2 of 1.0. The technique involves wedging a size 6F or 8F end hole suction catheter. A size 8 suction catheter was used for endotracheal tube size 3.5 mm internal diameter, and size 6 for 3.0 mm or smaller. The infant was positioned supine with the head turned 90° to the left; such a position virtually ensures that a catheter advanced down the trachea enters the right main bronchus. While ventilation continued, the catheter was introduced into the

endotracheal tube by way of a suction bullet in a swivel Y-connector, and gently advanced until met with resistance. An aliquot of 1 ml per kg body weight of saline, warmed to 37°C, was instilled through the catheter, coinciding with a positive pressure inflation of the lung. The dead space within the catheter was cleared with air after each injection. Low-pressure suction was then immediately applied (60 to 90 mm Hg for a size 8 catheter, 80 to 120 mm Hg for a size 6), and the return fluid collected in a mucus trap. A total of three aliquots were thus instilled, with suction after each.The fluid returned from all aliquots was pooled, and subsequently processed as described[8].

Temperature, heart rate, blood pressure, and oxygen saturation were monitored throughout the procedure. One hour after completion of the sampling, a complete clinical examination and a chest radiograph were systematically performed.

Contraindications to NB-BAL were the following: mechanical ventilation with F IO_2 >0.6, severe pulmonary interstitial emphysema, pneumothorax, bradycardia (heart rate <80 beats/min in neonates), hypotension (mean arterial pressure <40 mm Hg in neonates), and platelet count of <30,000/mm^3.

Cytologic Analysis

The cytologic study of NB-BAL was performed on a homogeneous sample. This sample was centrifuged for 10 min at 8,000 g, and the NB-BAL fluid was stained using Gram's and Wright stains. For NB-BAL specimens, a total cell count was performed on aliquots of resuspended NB-BAL using a hemocytometer counting chamber. Slides were stained with Wright and Gram's stains. The Gram's stain was examined at high magnification (x100) to determine the morphologic features of bacteria and the percentage of ICB (intracytoplasmic bacteria within macrophages and neutrophils) by counting 100 cells. To minimize the variability dependent on the operator's skill, all of the cytologic procedures were performed by the same microbiologist.

Bacteriologic Analysis

The specimen was cultured on sheep's blood, chocolate agar, and MacConkey plates and all bacterial species isolated were identified by standard microbiological technique. Each distinct colony was counted separately, multiplied by 10 and recorded as colony forming units per milliliter [(cfu)/ml] of BAL. We used 10^5 cfu/ml of BAL as the quantitative cut-off point for culture positivity.

Statistical Methods

Quantitative variables were expressed as mean±SD, and comparisons between the two groups of children (infants with VAP and infants with no VAP) were performed using the unpaired Student's t-test. Qualitative variables were expressed as observed numbers or proportions, and comparisons between infants with and without VAP were performed using the chi-square contingency test. In all statistical testing procedures, $p < .05$ was considered significant. Standard definitions were used for determining the sensitivity, specificity, and predictive values.

Results

Of the 145 neonates, 40 (27.5%) were infected and 12 (8.3%) were colonized. Forty-four patients (30%) developed VAP according to diagnostic categories based on clinical and radiologic criteria. Forty newborns with VAP (90%) had positive NB-BAL culture. The study group of 44 patients consisted of 14 girls and 30 boys. Eighty-two percent of this group were premature, with a mean gestational age of 30.2±3.8 weeks and birth weight of 1720±734 g. The newborns were on mechanical ventilation because of a variety of medical conditions. The diagnoses at admission of the 44 newborns with VAP were respiratory distress syndrome (n=37), meconium aspiration syndrome with persistent pulmonary hypertension (n=3), and hypoxic ischemic encephalopathy with persistent pulmonary hypertension (n=4). All babies received parenteral nutrition (PN). Mean duration of prior mechanical ventilation was 12.5±7.4 days. The duration of mechanical ventilation at this hospital stay ranged from 2 to 90 days, with a mean of 14 days and median of 8 days. Infants with VAP tended to receive mechanical ventilation for a longer period than those without VAP ($p<0.0001$). They also tended to require more manipulations, such as the placement of an umbilical catheter ($p<0.001$).

The general characteristics of children with and without VAP were similar (Table I). The incidence of VAP increased with decreasing gestational age. The risk factors for VAP were found to be prematurity, placement of umbilical catheter, PN, and longer duration of mechanical ventilation.

Airway colonization occurred in 8 (20%) neonates within the first 72 hours of life and in 32 (80%) during the following days. Antibiotic treatment was changed on the basis of NB-BAL culture results. Follow-up cultures of the NB-BAL were obtained in 16 neonates in whom antibiotics were changed.

Table I. Clinical Characteristics of the Newborns With and Without Ventilator-Associated Pneumonia (VAP)

Clinical Characteristics	VAP N:44	No VAP N:101	p
Gestational age (week)	30.2±3.8	33.4±3.8	<0.05
Gender (M/F)	30/14	61/40	NS
Birth weight (g)	1720±734	2090±1395	NS
Preterm (%)	36 (82%)	69 (68%)	NS
Duration of mechanical ventilation (days)	12.5±7.4	6.5±4.8	<0.0001
Parenteral nutrition	44	74	<0.001
Central venous catheter	44	78	<0.001

[a] Quantitative variables are expressed by mean ± SD. NS: non-significant.

Two hundred and seven NB-BAL samples were obtained from 145 intubated neonates for microbiologic and cytologic evaluation of the distal airway. Concomitant with each NB-BAL, clinical data and laboratory tests were recorded. One hundred and fifty-five (85%) NB-BAL samples were negative and 52 (15%) were positive. Among the positive cultures, 63% were Gram-negative bacteria, 31% were Gram-positive bacteria, and 6% were fungi. The infecting agent was isolated in 52 specimens. Acinetobacter baumannii was the causative agent in 14 (27%), Klebsiella sp in 11 (21%), Staphylococcus aureus in 9 (17.3%), Pseudomonas aeruginosa in 5 (9.6%), Staphylococcus epidermidis in 5 (9.6%), Candida albicans in 3 (5.8%), Stenotrophomonas maltophilia in 3 (5.8%), and Escherichia ictaluri in 2 (3.9%) (Table II).

Table II. Results of Nonbronchoscopic Bronchoalveolar Lavage (NB-BAL) Fluid Culture

	NB-BAL fluid culture	
Pathogen	N	%
A. baumannii	14	27
K. pneumoniae	11	20
S. aureus	9	17
P. aeruginosa	5	10
S. epidermidis	5	10
C. albicans	3	6
S. maltophilia	3	6
E. ictaluri	2	4
Total	52	100

Negative cultures were found in 11 of these neonates, and 83% of these cases showed clinical improvement.

Twenty seven percent of the infants with VAP developed positive gastric aspirate culture, 18% had a positive blood culture, and 9% had a positive urine culture. The same microorganism grown in eight of the positive blood cultures was also isolated in the NB-BAL culture. Most common isolates from blood were A. baumannii (n=3), K. pneumoniae (n=2), S. aureus (n=2) and S. aeruginosa (n=1) (Table III). Colonization occurred rapidly; organisms initially appeared in gastric aspirates (mean 2 days), then in tracheal aspirates (mean 5 days) and then urine cultures (mean 7 days). The acquired organisms, many of which were antibiotic-resistant, were almost exclusively enteric Gram-negative bacilli (GNB) and S. aureus.

Table III. Microorganisms Isolated in Newborns With Ventilator-Associated Pneumonia

Pathogen	NB-BAL culture	Bloodstream infection	Urinary tract infection	Stomach colonization
A. baumannii	9	3	1	3
K. pneumoniae	5	2	2	3
S. aureus	5	2		3
P. aeruginosa	5	1	1	2
S. maltophilia	2			1
E. ictaluri	2			

NB-BAL: Nonbronchoscopic bronchoalveolar lavage.

According to our diagnostic criteria, 44 newborns had VAP, and NB-BAL cultures were positive in 40 (90%) of them. Accordingly, the sensitivity of NB-BAL fluid culture for VAP diagnosis was 90%, specificity 90%, positive predictive value 70% and negative predictive value 97%.

In our study, the presence of leukocytes in the NB-BAL fluid smear of infants with VAP was higher than that of the colonized babies (84%, 26%). This difference was statistically significant ($p<0.0001$). The sensitivity and the specificity of polymorphonuclear cells (PMNs) in NB-BAL fluid for the diagnosis were, respectively, 86% and 75%; positive and negative predictive values were 89% and 69%. The percentage of PMN containing ICB was significantly higher (14.6 ± 4.7 versus 1.1 ± 3.4, <0.0001) in newborns with VAP. The presence of ICBs in 2% or more of the cells on the Giemsa stain corresponded to a sensitivity of 94%, specificity of 83%, positive predictive value of 94%, and negative predictive value of 83%. The sensitivity and specificity of combination of ICB and NB-BAL quantitative culture in sample for diagnosis were 94% and 90%, respectively, and the positive and negative predictive values were 71% and 98% (Table IV). Microscopic examination of NB-BAL fluid may help in differentiating tracheobronchial colonization and infection.

There were no significant complications associated with this method of NB-BAL. We determined a transient increase in systolic blood pressure in one newborn. This method was associated with transient arterial desaturation, but did not result in any prolonged compromise of gas exchange.

No arrhythmias, clinically significant pulmonary hemorrhage, or pneumothoraces occurred due to the procedure and no deaths were directly attributable to NB-BAL. The oxygenation index and ventilator setting were essentially the same before and after NB-BAL.

Discussion

Nosocomial pneumonia is a major cause of morbidity and mortality in hospitalized patients. The risk is especially high in the NICU, particularly in mechanically ventilated infants[1]. VAP is defined as a bacterial pneumonia that develops in a patient who has received mechanical ventilation for at least 48 hours. It is associated with a mortality rate of 33 to 71% despite treatment with antibiotics; this may be due to the virulence of the causative pathogens[11]. Despite an extensive experience with this disease, the accurate diagnosis of pneumonia remains confusing and difficult in mechanically ventilated patients. Therefore, several techniques have been developed to improve the accuracy of the diagnosis. Many diagnostic techniques, such as protected specimen brush[11], BAL[8], and protected BAL[12], performed with or without fiberoptic bronchoscopy, have been evaluated. There are some potential risks involved in performing bronchoscopies in ventilated patients, including temporary alterations in lung mechanics and gas exchange, barotrauma, increase in intracranial

Table IV. Comparison Between Sensitivity and Specificity of Microscopic Quantification of ICB in Macrophages and PMN By the Method of Sampling

	Sensitivity (%)	Specificity (%)
ICB in NB-BAL sample	94	83
NB-BAL fluid culture*	90	90
PMN in NB-BAL sample	86	75
Combination of ICB and NB-BAL quantitative culture*	94	90

*cfu/ml: Colony-forming units per milliliter. ICB: Intracellular bacteria. NB-BAL: Nonbronchoscopic bronchoalveolar lavage. PMN: Polymorphonuclear cells.

Five of the 44 patients (11%) died. In all five newborns who were receiving an empirical antimicrobial treatment at the time of their inclusion in the study, the cause of death was attributed directly to multi-resistant Gram-negative pneumonia and sepsis. Histologic examination and quantitative cultures of lung-biopsy specimens were consistent with NB-BAL results.

pressure, or cross-transmission of pathogens. Other reported complications have included arrhythmias, transient worsening in pulmonary infiltrates, and fever. There is some additional risk with specific procedures, including bleeding from the brush procedure or severe hypoxemia from the BAL procedure. These risks are also present for blind procedures, and the risk of hypoxemia is present even in collecting tracheal aspirate[6]. End-expiratory volume and positive end-expiratory pressure are reduced, facilitating alveolar closure and venous admixture. These changes slowly improved after the procedure[5]. Recently, NB-BAL has been described in ventilated infants and children[7], and such techniques have been performed in neonates, including premature infants weighing less than 1,500 g[13,14].

Marquette et al.[15] showed at least moderate specificity for noninvasive as well as invasive sampling techniques. NB-BAL is a very useful technique for investigating respiratory disorders of newborn infants. It is safe and easy to perform even in the sickest infants receiving mechanical ventilation[6].

In this study, 207 NB-BAL were obtained from the distal airway of 145 intubated neonates for microbiologic and cytologic evaluation. Of the 145 neonates, 40 (27.5%) were infected and 12 were colonized. Forty-four patients (30%) developed VAP according to diagnostic categories based on clinical and radiologic criteria. Forty newborns with VAP (90%) had positive NB-BAL culture. One hundred and fifty-five BAL were negative, while the 52 (15%) positive yielded Gram-negative bacteria (63%), Gram-positive bacteria (31%), and fungi (6%). Mas-Munoz et al.[16] reported coagulase-negative staphylococcus as the leading causative agent (45%), followed by K. pneumoniae (17%), in the etiology of pneumonia in mechanically ventilated patients. Brook et al.[17] also reported S. aureus as the most common agent. In contrast, we cultured A. baumannii in 27%, followed by Klebsiella[21] and S. aureus (17.3%).

In our study, the sensitivity of NB-BAL fluid culture for VAP diagnosis was 90%, specificity 90%, positive predictive value 70% and negative predictive value 97%. Balthazar et al.[18] had reported that the sensitivity of BAL fluid culture for VAP diagnosis was 77%, specificity 87%, positive predictive value 71%, and negative predictive value 90%. Our data suggest that NB-BAL culture has good sensitivity and high specificity for VAP diagnosis.

Recent studies have shown the usefulness of microscopic examination of the BAL fluid for early diagnosis of VAP. Gram and Giemsa stains identify most of the microorganisms that grow at significant concentrations, but their validity is a subject of controversy[3,4]. In the study by Rello et al.[19], the diagnosis of VAP was confirmed in only 46% of the cases. Furthermore, these tests did not distinguish between colonization and infection. In our study, the presence of leukocytes in the BAL fluid smear of infants with VAP was higher than that of the colonized babies (84%, 26%). This difference was statistically significant ($p<0.0001$). The sensitivity and specificity of PMNs in NB-BAL fluid for the diagnosis were 86% and 75%, respectively. We showed the percentage of ICBs was significantly higher in nosocomial pneumonia (14.6 ± 4.7 versus 1.1 ± 3.4) (<0.0001). The diagnostic accuracy of Gram's stain was at least as good as the examination of ICB in our series of patients. Unfortunately, few data are available on the usefulness of Gram's stain in BAL in case of VAP. Papazian et al.[20] have reported both the presence and absence of ICB. In this study, the presence of neutrophils containing bacteria was found highly specific (98.3%), but unfortunately not sensitive enough (19.2%). Allaouchiche and coworkers[21] reported the sensitivity (84%), specificity (80%), positive predictive value (69%), and negative predictive value (90%) of the presence of ICBs. In our study, we determined that the presence of ICBs in 2% or more on Giemsa stain corresponded to a sensitivity of 94%, specificity of 83%, positive predictive value of 94%, and negative predictive value of 83%. We found that the sensitivity and specificity of combination of ICB and PMN counts in NB-BAL sample for diagnosis were respectively 94% and 75%. The count of ICBs in NB-BAL fluid allows a rapid and accurate diagnosis of VAP. This technique is a sensitive and specific means for early and rapid diagnosis of pneumonia and allows specific treatment for most patients. In our opinion, the determination of ICBs in NB-BAL fluid may be considered as the reference method for the early diagnosis of

VAP. Microscopic examination of NB-BAL fluid may help to differentiate tracheobronchial colonization and infection. We found that the sensitivity and specificity of combination of ICB and NB-BAL quantitative culture in sample for diagnosis were 94% and 90%, respectively, and the positive and negative predictive values were 71% and 98%. Immediate diagnosis of VAP is possible with NB-BAL, and culture allows the identification of the causative microorganisms in the following days.

There were no significant complications associated with this method of NB-BAL. We observed a transient increase in systolic blood pressure in one newborn. Belai et al.[14] also reported a transient increase in systolic blood pressure in neonates. This method was associated with transient arterial desaturation, but did not result in any prolonged compromise of gas exchange. Transient arterial oxygen desaturation that readily resolves occurred in our babies, and this has been previously described by Dargaville[8] and Grigg[13]. NB-BAL is generally well-tolerated by critically ill mechanically ventilated newborns.

A. baumannii, K. pneumoniae and S. aureus were the most frequently isolated bacteria, especially in the 44 newborns with VAP. In such situations, therapeutic decisions based on clinical features, radiographic findings, and local epidemiology may be inadequate, because most of these bacterial isolates have significant antimicrobial resistance[22]. Inappropriate antimicrobial therapy has been shown to be strongly related to mortality. NB-BAL, which yielded 90% of the causative microorganisms in our study, may guide decisions concerning antibiotic choice.

We conclude that NB-BAL is well tolerated and clinically useful in mechanically ventilated newborns. This prospective study shows that NB-BAL is effective in collecting distal respiratory tract secretions, with a minor degree of contamination. These results suggest that NB-BAL fluid microscopic examination and cultures can offer a sensitive and specific means to diagnose VAP in newborns and may provide relevant information about the causative pathogens. In ventilator-associated pneumonia, NB-BAL was contributive to decision-making concerning antimicrobial therapy. Initiation of antibiotics may be guided by microscopic examination of NB-BAL. Later, the initial therapy can be modified, based on the results of NB-BAL quantitative cultures.

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