Diagnosis of Bacterial Pulmonary Infections with Quantitative Protected Catheter Cultures Obtained During Bronchoscopy

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Quantitative bacteriology was performed on specimens collected by protected catheter fiberoptic bronchoscopy from 172 patients. Of the patients who had pneumonia, 75 of 78 (96%) had one or more species present at ≥10³ CFU/ml, whereas 2 of 35 (6%) control patients had organisms present in that quantity. In addition, 66% of the control specimens yielded no isolates by this technique. All of the 11 patients with bronchitis had ≥10³ CFU/ml. Quantitative bacteriology revealed high levels of colonization in patients without infection and endobronchial structural disease. The data suggest that bacterial counts of ≥10³ CFU/ml in suspended secretions collected with a protected catheter brush were diagnostic of the bacteriological etiology of lower respiratory tract infections in patients without endobronchial structural abnormalities.

Many methods have been described for the etiological delineation of lower respiratory tract infections. Several modifications of standard methods have been suggested for improving culture results from expectorated sputum. These included quantitation of the entire homogenized specimen (6, 9, 11, 22), washing the specimens to eliminate saliva (6), and screening specimens for quality by Gram stain (8). Although these modifications represent improvements over routine processing, comparison with more definitive methodologies, such as blood cultures and transtracheal aspiration (TTA) revealed the limitations of these culture techniques in the evaluation of lower respiratory tract infections (2, 5, 7, 11, 17). Barrett-Connor (2) evaluated sputum cultures in the diagnosis of bacteremic pneumococcal pneumonia. In only 45% of the bacteremic patients studied was Streptococcus pneumoniae recovered from sputum cultures.

TTA has gained broad acceptance as a diagnostic tool for lower respiratory tract infections (21). This technique has been particularly useful in the delineation of anaerobic infections (5) and has been used to demonstrate the inadequacy of routine sputum cultures (5, 7, 11, 17). However, TTA is an invasive procedure involving some risk to patients. In addition, one study showed discrepancies between bacteria recovered by TTA and by transthoracic needle aspiration in dogs with experimental pneumonia (20). Other studies have isolated organisms other than the primary pathogen. The importance of these additional isolates has been difficult to ascertain without quantitative bacteriology (3, 14).

Several workers have studied the use of bronchoscopy as an alternative method for obtaining specimens for culture. Aspirates taken during routine bronchoscopy have been generally less reliable than TTA specimens due to contamination by oropharyngeal flora (4, 16, 21). Wimberley et al. (24) described a fiberoptic bronchoscopy technique which utilized a double lumen catheter with a brush and a polyethylene glycol plug. In vitro studies showed that the catheter was capable of accurate specimen sampling after passage through a contaminated bronchoscope. Subsequent studies with both healthy volunteers and patients showed that lower respiratory tract specimens could be obtained with minimal oropharyngeal contamination (12, 23, 24). These studies suggested that quantitative bacteriology was necessary for proper interpretation of culture results and further suggested that a colony count of ≥10³ CFU/ml could be used as an interpretative breakpoint for determining the significance of an isolate.

We tested the usefulness of quantitative cultures of protected catheter bronchoscopy (PCB) specimens over a 3-year period (23). PCB culture results were correlated with clinical evidence of pulmonary infection and blood cultures. In addition, results of sputum and
bronchial wash cultures were compared with the new technique.

MATERIALS AND METHODS

Patient population. In the 3-year period from May 1978 to May 1981, PCB was performed on 172 patients. Twenty-eight patients were eliminated from the study because they had received antibiotics before bronchoscopy; this left a study group of 144 individuals. Lower respiratory tract infections (bronchitis and pneumonia) were diagnosed in 89 of the study patients. The diagnosis of pneumonia in 78 patients was based on typical clinical findings and response to appropriate antibiotics. The diagnosis of bronchitis in 11 patients was defined as severe exacerbation of preexisting chronic lung disease, with an increase in purulent sputum production, plus bronchoscopically visible mucosal inflammation in patients without roentgenographic infiltrates. Thirty-five patients with no underlying endobronchial disease and no evidence of lower respiratory tract infection had PCB performed during routine bronchoscopy and served as a control group. Twenty patients undergoing bronchoscopy for the diagnosis of noninfectious endobronchial disease had PCB performed to study bacterial colonization in patients with severe alterations in bronchial anatomy. These alterations included central bronchogenic carcinoma (13 patients), tuberculous bronchiectasis (4 patients), and endotraheal sarcoidosis (3 patients).

PCB. The patients were premedicated intramuscularly with atropine and meperidine. Tracheobronchial anesthesia was obtained by nebulizing lidocaine without methylparaben preservative (24, 25). Topical anesthesia was obtained by nebulizing 10 to 15 ml of 4% lidocaine through a mouthpiece over a 15 to 20 min period with a disposable plastic nebulizer giving a particle size of 0.5 to 5 µm in diameter. To avoid contamination from upper airway flora, no topical anesthetics were injected through the bronchoscope channel nor was suction applied before obtaining the specimen. Transnasal bronchoscopy was done with the patients sitting upright. The bronchoscope tip was positioned adjacent to directly visualized secretions; when secretions were not visible, samples were obtained from the roentgenographically abnormal lobe or bronchopulmonary segment. In the control group, the lower trachea was sampled. The specimens were obtained within 5 min after completion of the nebulization procedure with a telescoping cannula brush catheter with a polyethylene glycol plug (BFW brush; MediTech Corp., Watertown, Mass.) (24). After the specimen was obtained, the entire brush unit was removed from the bronchoscope. A small amount of the material obtained was smeared onto a sterile glass slide for Gram staining, and the brush was aseptically cut off and placed in a vial containing 1.0 ml of Ringers lactate solution.

PCB cultures. The vial containing the brush and Ringers lactate solution was placed on a Vortex mixer to thoroughly suspend all material from the brush. Two serial 10-fold dilutions were made, and 0.1 ml of the original suspension and each dilution was placed on each of six agar plates. The resulting cultures represented 10⁷, 10⁵, and 10³ dilutions of the original specimen suspended in Ringers lactate. Since the quantity of collected secretions was not uniform, no attempt was made to account for the initial dilution, which ranged from 100 to 1,000-fold the specimen in Ringers lactate (24). Media for aerobic cultures included blood agar base with 5% sheep blood, MacConkey agar, and GC-agar base with 5% chloretone sheep blood and 1% IsoVitaX (BBL Microbiology System) incubated in CO₂ at 35°C. The anaerobic media included brucella agar base with 5% sheep blood, hemin (5 µg/ml), and vitamin K (10 µg/ml); laked blood with vitamin K (10 µg/ml), kanamycin (100 µg/ml), and vancomycin (7.5 µg/ml); and Shaded agar base with 5% sheep blood, colistin (100 µg/ml), and nalidixic acid (150 µg/ml) incubated in GasPak jars (BBL). All media were poured by Professional Laboratory Services, Birmingham, Ala. Bacterial identification was done by standard methods (19).

Bronchial wash and sputum cultures. Bronchial washings were obtained by lavage through the bronchoscope channel with instillation of 10 to 30 ml of normal saline after PCB was performed. Five to ten milliliters of material was recovered. Expectorated sputum were initially screened for cellular content. Expectorated sputa and the bronchial wash specimens were qualitatively cultured on blood agar base with 5% sheep blood, MacConkey agar, and GC-agar base with 5% chloretone sheep blood and 1% IsoVitaX. All media were incubated in CO₂ at 35°C.

Specimen comparisons. There were 58 patients with pneumonia for whom comparisons between PCB and sputum or bronchial washes could be made. The remaining 16 patients with mixed aerobic-anaerobic pneumonia were not examined since these infections cannot be diagnosed by sputum and bronchial washes owing to upper airway contamination.

RESULTS

The PCB culture results were examined to determine whether there was a correlation between ≥10³ CFU/ml for one or more species and clinical evidence of lower respiratory tract infection. Among the patients presenting with bacterial pneumonia, 75 of 78 (96%) had ≥10³ CFU of one or more bacterial species per ml isolated from their PCB cultures (Table 1). One of the patients with <10³ CFU/ml had a primary pathogen isolated and bacteremia caused by the same organism. The other two patients had no organism isolated; one patient had an enclosed ab-

<p>| TABLE 1. Comparison of bacterial counts ≥10³ CFU/ml for one or more species in patients with clinical evidence of lower respiratory tract infection |
|-----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Clinical picture</th>
<th>No. of patients</th>
<th>With bacterial</th>
<th>Counts (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>75</td>
<td>3</td>
<td>10⁷</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>11</td>
<td>0</td>
<td>10⁵</td>
</tr>
<tr>
<td>Controls (no infection)</td>
<td>2</td>
<td>33</td>
<td>10³</td>
</tr>
<tr>
<td></td>
<td>≥10³</td>
<td>&lt;10³</td>
<td></td>
</tr>
</tbody>
</table>

J. CLIN. MICROBIOL.
TABLE 2. Bacterial species isolated at ≥10³ CFU/ml from cases of pneumonia

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. of isolates</th>
<th>Isolated as:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Single agent</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>38</td>
<td>24</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Other isolate</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Aerobic-anaerobic</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>mixed flora without a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>traditional pathogen</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁺ Ten of these had anaerobes.

scess, and the other case was presumed to be Legionnaires disease because it responded to erythromycin. Chi-square analysis indicated that ≥10³ CFU/ml for one or more species was statistically more common (P < 0.001) in patients with pneumonia than in controls (2 of 35 (6%)) (Table 1). S. pneumoniae was the most common primary pathogen; it was present with significant numbers of other species in 14 of 38 (37%) cases. Haemophilus influenzae was infrequently found in pure culture, with other isolates being present in significant numbers 71.4% of the time (Table 2). Four patients had mixed infections, with S. pneumoniae and H. influenzae being present in significant counts. Specimens from 71% of the 14 patients from whom S. pneumoniae was isolated with mixed flora and from 90% of the 11 patients from whom H. influenzae was isolated with mixed flora had anaerobes recovered in significant numbers. The most common anaerobic species observed included normal oral flora, with Bacteroides melaninogenicus being the most common anaerobe present in significant numbers (Table 3). The other organisms present in significant numbers were Staphylococcus aureus in two cases, and Neisseria sp. was present at 10³ CFU/ml in one patient with clear evidence of pneumonia. Sixteen patients had significant counts of mixed aerobic and anaerobic flora without any commonly recognized pathogen. The spectrum of species represented in significant numbers was similar to that associated with mixtures of flora observed with commonly recognized pathogens (Table 3).

Thirteen patients with pneumonia had positive blood cultures. Of these, 12 had significant counts (≥10³ CFU/ml) of the blood isolate in the PCB, and 1 had <10³ CFU/ml for the reported blood isolate. Six of seven bacteremic cases had the documented blood isolate in the bronchial wash culture, whereas two of six patients had sputum cultures yielding the blood isolate.

There were 11 bronchitis patients in this series, all of whom had at least one organism in amounts ≥10³ CFU/ml that grew from their PCB. H. influenzae was the predominant organism isolated from eight patients. It was present in pure culture in specimens from four patients and with mixed aerobic flora in the other four. Only one of these PCB specimens had four anaerobic species present; three of the four species were present in significant numbers, and the fourth was B. melaninogenicus (10³ CFU/ml). There were two cases with Pseudomonas aeruginosa present in pure culture, and one case with S. pneumoniae present with mixed flora.

Specimens from the control group (Table 1) yielded significant counts in all but two cases. Each of these two patients had an underlying malignancy; one had oropharyngeal cancer and the other had lymphoma. The significant counts of viridans streptococci were at the 10³ CFU/ml breakpoint in both cases. Of the control specimens, 23 of 35 (66%) had no growth from the cultures, representing a count of <10¹ CFU/ml on the diluted secretions.

In addition to the above groups, PCB cultures were taken from 20 patients with structural endobronchial disease. Of these 20, 11 (55%) had ≥10³ CFU/ml, 7 of which were mixed aerobic-anaerobic flora; 3 had H. influenzae plus other organisms, and 1 had a Corynebacterium sp.

There were 58 patients for whom there was a recognized aerobic pathogen that could be evaluated for isolation of the pathogen from different types of specimens (Table 4). Nineteen patients had routine sputum specimens accompanying the PCB. Eighteen of those for whom a sputum culture was available had ≥10³ CFU/ml for a recognized pathogen by PCB, with only 6 of the 18 having the pathogen isolated by sputum culture. Thirty-three patients from whom a bronchial wash was available had ≥10³ CFU/ml for a recognized pathogen from PCB, and 23 of these had the same organism isolated from the bronchial wash (Table 4).

TABLE 3. Anaerobic species associated with mixed flora pneumonias

<table>
<thead>
<tr>
<th>Organism</th>
<th>Isolated with a primary pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (21 patients)</td>
</tr>
<tr>
<td>Bacteroides melaninogenicus</td>
<td>12</td>
</tr>
<tr>
<td>Fusobacterium spp............</td>
<td>5</td>
</tr>
<tr>
<td>Veillonella spp..............</td>
<td>2</td>
</tr>
<tr>
<td>Bacteroides ruminicola......</td>
<td>3</td>
</tr>
<tr>
<td>Bacteroides oralis...........</td>
<td>5</td>
</tr>
<tr>
<td>Peptococcus spp..............</td>
<td>3</td>
</tr>
<tr>
<td>Peptostreptococcus spp.......</td>
<td>2</td>
</tr>
<tr>
<td>Bacteroides spp..............</td>
<td>5</td>
</tr>
</tbody>
</table>
Gram stain results for specimens from the pneumonia cases were examined to determine the number of times the organism isolated by PCB culture at $\geq 10^5$ CFU/ml was represented by a comparable Gram reaction and morphology on direct smear of the PCB specimen. For 47 of 68 specimens, all of the organisms isolated in significant numbers were seen on Gram stain, or the smear was negative when the organisms were present in insignificant numbers. An additional six smears showed some but not all of the organisms in significant quantity on culture. The remaining 15 smears failed to show significant isolates.

**DISCUSSION**

PCB was developed as a method for sampling lower respiratory tract secretions near the site of their production while minimizing upper airway contamination of the sample. In addition, the use of quantitative bacteriology with the technique enhances its ability to distinguish between significant and insignificant isolates. The data presented here show the great reliability of this approach. Although there have been other reports on fiberoptic bronchoscopy (10, 16, 18, 21), aspirates drawn through the bronchoscope appeared to be contaminated with upper airway flora (16, 21). Evidence for the lack of upper airway contamination at the initial dilution in the present study was given by the 23 specimens from control patients that had no organisms isolated from culture. Although the possibility of airway contamination in patients with pneumonia and not in controls cannot be absolutely discounted, the known tracheobronchial clearance rates for bacteria make this an unlikely explanation for the data. In addition, control patients were sampled at more proximal sites that were more likely to be contaminated. The only group of patients for whom quantitative PCB cultures were of limited value were those with structural endobronchial disease, which apparently led to colonization with large quantities of bacteria in approximately one half of the cases.

Wimberley et al. (24) tentatively suggested an interpretative breakpoint for PCBs of $\geq 10^3$ CFU/ml for determining the significance of an isolate. Since the initial specimen was diluted 100 to 1,000-fold in Ringers lactate, this cut-off actually represents $\geq 10^2$ to $10^6$ CFU/ml in the original secretions. This is similar to the level reported for TTA (14). This proposed interpretation was confirmed in our group of patients, studied over a 3-year period. Among the patients with pneumonia, there were a large number with mixed flora, including anaerobes, in their PCB specimens (Table 2). Therapeutic evidence suggested that the pneumonias with a primary pathogen, such as *S. pneumoniae*, from which significant numbers of anaerobic flora such as *B. melaninogenicus* were isolated behaved more like mixed flora pneumonias, requiring longer-term therapy than is commonly used for pneumococcal pneumonia (J. Bass, E. Hawkins, J. R. Bonner, and H. M. Pollock, submitted for publication). Bartlett (3), using TTA, reported a similar proportion of patients with mixed flora accompanying a primary pathogen. He suggested the use of quantitative cultures with transtracheal aspirates for delineating their significance (3). Irwin et al. (14) reported the usefulness of this quantitative modification for TTA (14).

PCB as a method for specimen collection appears to be as good as TTA and possibly superior (20). Moser et al. (20) described an experimental study with dogs in which a variety of collection procedures were compared. PCB and TTA were among those described, and PCB appeared to be more sensitive and specific than TTA as measured against transthoracic needle aspirates.

The cases of bronchitis were remarkable by the apparent lack of significant quantities of anaerobes in all but one case. These were similar to the results reported by others with TTA (15, 16).

Bronchial wash and sputum specimens were much less sensitive compared with PCB. Sputum specimens diagnosed only 6 of 18 pneumonias caused by a primary pathogen. Many physicians believe that bronchial washes give better results than sputum does. Although the results presented here indicate that bronchial washes increased the sensitivity of isolating primary aerobic pathogens, upper oropharyngeal contamination interfered with interpretation and prohibited use of the technique for delineating the mixed flora nature of lower respiratory tract infections (4, 14, 20).

The Gram stain of the PCB proved to be a good predictor of culture results and was 78% sensitive.

Quantitative PCB has been described in comparison with the clinical status of each of the patients studied. It was found to be very sensitive and specific for delineating the bacterial
etiology of pneumonia and bronchitis. In addition, it was useful for those patients who could not produce an expectorated sputum with which to make a diagnosis. The method also allowed the appreciation of the mixed flora nature of pneumonias that, by other methods, might have appeared to be pure S. pneumoniae or H. influenzae pneumonias. The data confirmed the thesis that organisms present at \( \geq 10^3 \) CFU/ml were clinically significant, whereas those present at <10^3 CFU/ml represented levels of bacterial flora associated with colonization in patients who do not have endobronchial structural alterations.

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LITERATURE CITED


