Quantitative Cultures of Endotracheal Aspirates for the Diagnosis of Ventilator-associated Pneumonia

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Bronchoalveolar lavage (BAL) and protected specimen brushing (PSB) are the most commonly used methods for diagnosing ventilator-associated (VA) pneumonia although they require bronchoscopy. Endotracheal aspiration (EA) is a simple and less costly technique than PSB or BAL. The purpose of our study was to investigate the diagnostic value of EA quantitative cultures and to compare the results obtained using EA with those obtained using PSB and BAL in mechanically ventilated patients with or without pneumonia. We prospectively studied 102 intubated patients divided into three diagnostic categories: Group I (definite pneumonia, n = 26), Group II (uncertain status, n = 48), and Group III (control group, n = 28). All patients received prior antibiotic treatment. EA, PSB, and BAL were obtained sequentially in all patients. When comparing Group I with Group III and using 10^6 cfu/ml as a threshold, we found that EA quantitative cultures represented a relatively sensitive (70%) and relatively specific (72%) method to diagnose VA pneumonia. The specificity of BAL and PSB (67% and 93%, respectively) was better than that of EA. The negative predictive value of EA cultures was higher (72%) when compared with that obtained using PSB (34%) (p < 0.05). EA quantitative cultures correlated with PSB and BAL quantitative cultures in patients with definite pneumonia. Although EA quantitative cultures were less specific than PSB and BAL for diagnosing VA pneumonia, our results suggest that the former approach may be used to treat these patients when bronchoscopic procedures are not available. El-Ebiary M, Torres A, González J, de la Bellacasa JP, García C, de Anta MTJ, Ferrer M, Rodriguez-Roisin R. Quantitative cultures of endotracheal aspirates for the diagnosis of ventilator-associated pneumonia. Am Rev Respir Dis 1993; 148:1552–7.

METHODS

Patients

We studied 102 mechanically ventilated (for > 72 h) patients with acute respiratory failure who were admitted to the Respiratory Intensive Care Unit (UVIR) of a 1,000-bed teaching hospital. Patients were classified into three diagnostic categories. In Groups I and II, patients were entered into the study when there was a clinical suspicion of pneumonia. These patients were later assigned to the proper diagnostic category. Group I (definite pneumonia, n = 26). Patients who had at least one of the following criteria: (1) Positive pleural fluid or blood cultures for the same microorganism isolated from respiratory secretions; (2) Compatible histopathologic or necropsic findings with pneumonia; or (3) Isolation of definitive respiratory pathogens, such as Legionella, Mycobacterium tuberculosis, or Mycoplasma pneumoniae.

Ten patients (38.4%) were included on the basis of necropsy findings. Fifteen (57.7%) had positive blood cultures; in eight of these patients, this high percentage of false-positive results (4, 5) due to the bacterial colonization of the airways. However, very little information has been provided regarding the diagnostic value of EA quantitative cultures. Studies using semi-quantitative and quantitative cultures (6–9) suggest that EA samples have at least an acceptable negative predictive value on the one hand, and even a reasonable overall diagnostic accuracy on the other. The purpose of our study was to investigate the diagnostic value of EA quantitative cultures and compare the results obtained using this technique with those obtained using PSB and BAL in well-defined populations of mechanically ventilated patients with or without pneumonia.
was the only sure diagnostic method. Two patients had positive pleural fluid culture, two had positive serology for *Legionella*, and four yielded positive cultures of BYCE-a for *Legionella*.

Group II (uncertain status, n = 48). Patients who had new and/or progressive pulmonary infiltrates in addition to one of the following criteria: (1) Purulent respiratory secretions; (2) Temperature rise > 38.3°C (6); (3) Increase of ≥ 25% of circulating leukocytes. Alternative explanation of fever and/or leukocytosis on an extra-pulmonary basis was not accepted.

Group III (control, n = 28). Patients who had neither chest X-ray findings, leukocytosis, nor fever. These patients were entered into the study when they remained mechanically ventilated for > 72 h and met all the criteria mentioned above.

The main clinical findings and underlying conditions of the patients are shown in tables 1 and 2, respectively.

**Protocol**

The following protocol was performed in the same sequence in all cases. Permission of the Research Committee of the hospital was obtained.

First, EA were obtained by sterile means using a 22-in, No. 14 Fr suction catheter and collected in a mucus collector (Mocstrap®; Proclinics, Barcelona). Then, without interrupting mechanical ventilation, through the endotracheal tube and using a special adaptor, the fiberoptic bronchoscope (BFT3R; Olympus, New Hyde Park, NY) was introduced 1 h later without any bronchial suctioning after adequate sedation (nitrazepam, 1 mg) and curarization (pancuronium bromide, 4 mg). The setting of the ventilator was adapted appropriately during the procedure to ensure proper ventilation and oxygenation. Local anesthetics were not used. Finally, a PSB (BW 1/0/70/90; Medi-tech Inc., Watertown, MA) sample was retrieved from the middle lung lobe in patients without pneumonia and from the area of maximal local inflammation and purulence in patients with chest X-ray infiltrates, following a standardized technique previously described elsewhere (4, 10). After PSB sampling, the fiberoptic bronchoscope was wedged in one of the segments of the same area where PSB was performed. Two aliquots of sterile normal saline (50 ml each) were instilled and aspirated. The fluid obtained from the first 50 ml was discarded, and only the fluid obtained from the second aliquot was used for microbiologic processing. The mean BAL fluid obtained for processing was 15 ± 7 ml. Before the protocol procedures, two blood samples were taken for culture in each patient. PSB procedure was always performed before BAL sampling to avoid bacterial contamination from the bronchoscope channel. Necropsy studies were performed on 12 patients who died, after obtaining written permission from family.

**Microbiologic Processing**

Endotracheal samples were mechanohomogenized using glass beads and were vortexed for 1 min. PSB were aseptically cut into a sterile tube containing 1 ml of Ringer's lactate and vortexed for 1 min. The BAL fluid obtained was homogenized using repeated aspirations with a Pasteur's pipette. Serial dilutions (0.1, 0.01, and 0.001) of each sample were prepared in sterile normal saline. One hundred milliliters of each dilution of EA, PSB, and BAL were inoculated into the following agar media: 5% sheep blood, chocolate, Wilkins-Chalgren, CCO, Enko, BCYE-a, and Sabouraud-dextrose. All cultures were incubated at 37°C under aerobic and anaerobic conditions and in CO₂-enriched atmosphere. Cultures were evaluated for growth 24 and 48 h later and were discarded, if negative, 5 d after, except for CDC and Wilkins-Chalgren, which were evaluated at 7 d, and Sabouraud at 4 wk. Gram's and Ziehl-Neelsen stains and direct immunofluorescence against *Legionella pneumophila* were performed using undiluted samples. All microorganisms isolated were identified by standard laboratory methods. Results were expressed as cfu/ml = number of colonies x dilution factor x inoculation factor. Serologic tests for *L. pneumophila*, *M. pneumoniae*, *Coxiella burnetti*, and *Chlamydia psittaci* were performed in all the patients. Postmortem pathologic criteria

### Table 1

<table>
<thead>
<tr>
<th>PRINCIPAL CLINICAL FINDINGS OF THE POPULATION*</th>
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<tr>
<td>Group I: Definite Pneumonia (n = 26)</td>
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<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Mechanical ventilation period, d</td>
</tr>
<tr>
<td>Temperature, °C</td>
</tr>
<tr>
<td>SAPS</td>
</tr>
<tr>
<td>PaO₂/FIO₂</td>
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<tr>
<td>Average No. of antibiotic therapy, d</td>
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<td>Duration of antibiotic therapy, d</td>
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*Definition of abbreviation: SAPS = simplified acute physiology score.*

Results are expressed as mean ± SO.

### Table 2

<table>
<thead>
<tr>
<th>UNDERLYING CONDITIONS OF THE POPULATION, N (%)</th>
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<tbody>
<tr>
<td>Group I: Definite Pneumonia (n = 26)</td>
</tr>
<tr>
<td>COPD</td>
</tr>
<tr>
<td>Trauma</td>
</tr>
<tr>
<td>Surgery</td>
</tr>
<tr>
<td>Blood disorders</td>
</tr>
<tr>
<td>Neurologic disease</td>
</tr>
<tr>
<td>Cardiac disease</td>
</tr>
<tr>
<td>Pulmonary disease</td>
</tr>
<tr>
<td>other than COPD</td>
</tr>
<tr>
<td>Systemic hypertension</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
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<tr>
<td>Diaphragmatic paralysis</td>
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<tr>
<td>Others</td>
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*Definition of abbreviation: COPD = chronic obstructive pulmonary disease.*

### Table 3

<table>
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<tr>
<th>QUALITATIVE MICROBIOLOGIC ASSESSMENT</th>
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<tr>
<td>Group I: Definite Pneumonia</td>
</tr>
<tr>
<td>EA</td>
</tr>
<tr>
<td>Positive cultures*, n</td>
</tr>
<tr>
<td>Isolates, n</td>
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<tr>
<td>Polymicrobial cultures, n</td>
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*Presence of microbial growth independent of its quantification.*
of pneumonia included consolidated foci and polymorphonuclear leukocyte accumulation in bronchioles and adjacent alveoli (10).

Statistical Analysis

Results are expressed as mean ± SD. Student's t test was used to compare means. McNemar's test was used to compare diagnostic values of different techniques using different thresholds. Correlations were tested by linear regression analysis. Bacterial indices were calculated using the criteria described by Johanson and coworkers (11). This index is obtained by adding the logarithms of all the quantitative microbial cultures of each single sample. Sensitivity, specificity, predictive values, and diagnostic yields were calculated according to standard formulae (12), taking into account the individual species of microorganisms (13). Graphic representation of diagnostic parameters was performed, plotting true-positive against false-positive percentages to obtain a receiver operator characteristic (ROC) curve (14).

RESULTS

Qualitative Microbiologic Assessment

Table 3 shows the number of positive cultures and the total number of microorganisms isolated from each sampling technique as well as the number of the polymicrobial cultures.

**Group I (definite pneumonia).** Twenty-two of 27 (81%) microorganisms isolated from PSB samples were concomitantly isolated in EA samples. All microorganisms isolated from BAL samples were also cultured in EA samples.

**Group II (uncertain status).** Thirty-six of 41 (88%) microorganisms isolated in PSB samples were concomitantly isolated in EA samples. Fifteen of 21 (72%) microorganisms isolated from BAL samples were also cultured in EA samples.

**Group III (control).** Sixteen of 19 (84%) microorganisms isolated in PSB samples were concomitantly isolated in EA samples. Twenty of 20 (100%) microorganisms isolated from BAL samples were also cultured in EA samples.

Quantitative Microbiologic Analysis

Figure 1 shows the individual and mean bacterial concentrations of isolates from each sampling technique in the different diagnostic groups. Geometric means in Group I (definite pneumonia) were $10^{3.3}, 10^4$, and $10^{3.5}$ cfu/ml$^{-1}$ for EA, PSB, and BAL, respectively. For Group II (uncertain status), the means were $10^{3.4}, 10^{3.4}$, and $10^{3.5}$ cfu/ml$^{-1}$ for EA, PSB, and BAL, respectively. Means for Group III (control) were $10^{3.8}, 10^{3.5}$, and $10^{3.4}$ cfu/ml$^{-1}$ for EA, PSB, and BAL, respectively. Notably the geometric means of EA cultures were higher than those of PSB or BAL. For Group III (control) the geometric means of the three sample cultures were similar but lower than those of Group I, except for BAL.

Table 4 shows the correlation coefficients between quantitative cultures of coincident microorganisms isolated from different sampling techniques. In Group I we obtained acceptable and significant correlations between EA and PSB or BAL. Correlation between EA and PSB in group II (uncertain status) was not statistically significant ($r = 0.5, p = \text{NS}$). Correlations were also weak, yet statistically significant, in Group III (control) between EA and BAL cultures ($r = 0.6, p = 0.008$). When Groups I and II were analyzed together, correlations did not improve.

The geometric means, obtained from quantitative results of the bacterial indices, are shown in Table 5. Overall bacterial indices from EA were greater than those obtained from PSB and BAL. Interestingly, EA, PSB, and BAL geometric means of bacterial indices in patients with pneumonia were not significantly different from controls, indicating poor specificity of this parameter to distinguish colonization from infection.

Diagnostic Value

The diagnostic value of the different techniques according to different thresholds (analyzing Groups I and III) of quantitative cultures is shown in Figure 2. For EA, the best sensitivity/specificity ratio (72/70) was obtained using $10^5$ cfu/ml$^{-1}$. With the same threshold, the negative predictive value was 71%. As for PSB and BAL, $10^2$ and $10^3$ cfu/ml$^{-1}$ were the best thresholds, respectively. The sensitivity/specificity ratios for these techniques were 60/93 and 57/87 for PSB and BAL, respectively. Using the above-mentioned thresholds, the diagnostic yield of the three techniques was similar and without significant differences (71, 64, and 67%, respectively). When analyzing Group II versus Group III, sensitivity and specificity of the three techniques worsened, although EA diagnostic accuracy was similar to that of PSB and BAL (EA sensitivity 56%, specificity 65%, and diagnostic accuracy 62%; PSB sensitivity 52%, specificity 70%, and diagnostic accuracy 61%; BAL sensitivity 47%, specificity 74%, and diagnostic accuracy 60%). The ROC curve of the results obtained from Groups I and III is displayed in Figure 3. The best thresholds for quantitative cultures for each technique were $10^3, 10^2$, and $10^4$ cfu/ml$^{-1}$ for EA, PSB, and BAL, respectively.
Figure 2. Diagnostic accuracy parameters using \(10^5\), \(10^3\), and \(10^4\) cfu/ml as thresholds for EA, PSB, and BAL, respectively. The different diagnostic accuracy parameters were obtained comparing Groups I versus Group III. PPV = positive predictive value; NPV = negative predictive value; diag. yield = diagnostic yield.

Modification of Antibiotic Treatment

EA results promoted antibiotic treatment modification in 10 of 26 (38%), four of 48 (8%), and one of 24 (4%) patients from Groups I, II, and III, respectively. PSB results promoted a change in antibiotic therapy in eight patients (33%) from Group I. BAL results promoted antibiotic change in eight cases (33%).

DISCUSSION

The most important finding of the present study is that, when using \(10^5\) cfu/ml as a cut-off point, quantitative cultures of EA samples may be used for diagnosing VA pneumonia, although they cannot replace bronchoscopic techniques such as PSB and BAL, which are more specific. In patients with definite pneumonia, an acceptable correlation between EA and PSB or BAL cultures was obtained. A negative predictive value of 72% in EA samples was also a remarkable finding. Our results suggest, therefore, that EA quantitative cultures can be used, at least in part, as a diagnostic tool in the critical care setting, where fiberoptic bronchoscopic techniques are not always available 24 h a day.

It has been demonstrated that both PSB and BAL are valid methods for diagnosing nosocomial pneumonia in mechanically ventilated patients (1, 4, 15), and in many intensive care units these methods are commonly used. Traditionally, the common belief is that EA cultures are nonspecific methods to diagnose lower respiratory tract infection (LRTI) due to the colonization of both lower and upper airways. Using qualitative assessment of EA cultures, several investigators have demonstrated that this concept may not be necessarily true (4, 5, 16, 17). In two studies regarding the diagnosis of LRTI in mechanically ventilated patients, our group found specificities of 14% (4) and 29% (5). By obtaining pulmonary biopsies \textit{in vivo} in patients with acute respiratory failure, Hill and colleagues (17) found a lack of correlation between bronchial aspirate and biopsy cultures. In the present study, there was a qualitative agreement, ranging from 79 to 100%, among EA, PSB, and BAL cultures. Although some groups still use qualitative EA cultures for clinical management of patients with VA pneumonia (18), in view of the results alluded to above, there is no doubt about the poor specificity of this technique.
Over the last 5 yr, several investigators have tried to improve bronchoscopic procedures used to diagnose LRTI in mechanically ventilated patients. Surprisingly, many of them found acceptable results using methods that did not take bronchial secretion samples close to the inflammatory area of infection. Blind specimens taken via protected or unprotected BAL (19, 20) or PSB (5) have invariably demonstrated the accuracy of these methods. These findings indicate that pulmonary infection is a dynamic process, not restricted exclusively to the injured area of pneumonia. The latter statement was demonstrated 30 yr ago in an animal model developed by Pecora and Kohl (22), who retrieved by transtracheal aspiration the same microorganisms after 8 min of transpulmonary inoculation of *Serratia* spp.

We postulated that the quantitative cultures of simple EA samples might be useful in diagnosing VA pneumonia. Accordingly, we confirmed this hypothesis, obtaining a reasonable diagnostic accuracy (72%), sensitivity (70%), specificity (72%), and negative predictive value (72%) for this technique in patients with definite pneumonia, compared with controls. When patients with uncertain pneumonia were compared with controls, sensitivity and specificity of the three techniques worsened about 20 to 15%, but the overall diagnostic accuracy was comparable. These results, although not as good as those of PSB or BAL, could be considered acceptable for the routine clinical treatment in the absence of fiberoptic bronchoscopic facilities. Only a few authors have studied EA quantitative cultures (6–9, 13). One study found similar results to ours, establishing 10³ *cfu/ml* as the cut-off point for EA cultures to distinguish colonization from infection (6). In a previous study regarding control patients with mechanical ventilation (13), we demonstrated that 10³ *cfu/ml* was an inaccurate cut-off for EA cultures to be used confidently for the diagnosis of VA pneumonia. However, in another study (23) the same group obtained a specificity identical to that of the present study. Papazian and associates (8) compared PSB and EA quantitative cultures in 35 mechanically ventilated patients with bronchopneumonia and showed 93% agreement between the microorganisms cultured from both samples. Similar results were found by Baigelman and colleagues (7). However, these authors did not mention the cut-off point of EA cultures to distinguish colonization from infection; besides, a gold-standard technique was not available. Borderon and coworkers (9), comparing immediate pulmonary puncture cultures to EA quantitative cultures, showed that microbial species present in EA at concentrations ≥ 10³ *cfu/ml* were found in 53% of lung biopsies. Nevertheless, the predominant bacteria found in lung biopsies were not similar to those isolated from transthecal secretions. Other authors (24) suggest that EA cultures are useful immediately after intubation only. Indeed, a study comparing lung biopsy and EA cultures has not yet been performed. A clinical and practical consideration is the negative predictive value of EA cultures found in this study. A negative EA culture would probably rule out pulmonary infection. Similar considerations were stated by Richard and colleagues (25). Finally, a recent publication by Middleton and associates (26), comparing EA, ACCUCATH, PSB, and BAL for assessing VA pneumonia, showed a reasonable diagnostic value of Gram's stains and semi-quantitative cultures of EA.

There are several factors to consider in the present study concerning the diagnostic value of EA quantitative cultures. First, all the patients had been under antibiotic treatment, a fact that alters both the sensitivity and the specificity of the cultures (10, 11). Second, and coinciding with most studies of this type, there is no gold-standard technique, particularly in the control group. That means that we are not definitely sure that all control group patients were free from pneumonia. We believe that a study comparing microbiology and histopathology of immediate postmortem pulmonary biopsies in patients, with and without antibiotics, could be essential to explore these aspects. The third consideration refers to the primary pathogens isolated in the pneumonia group. In the latter, quantitative cultures are not necessary. The inclusion of these results in the analysis might therefore modify the established sensitivity. However, the reanalysis of sensitivities excluding these pathogens did not significantly change the final results (64 versus 70%). Finally, the methodology used to select the different groups of patients in the present study differs significantly from that of previous studies (1–5, 9), and this could have modified the results. In the majority of studies, patients were selected according to the presence or absence of pneumonia; intermediate categories or uncertain status were not taken into account. However, we have included in the present study one more category (uncertain status) that might help to obtain a broad patient selection.

As for bacterial indices, our results indicate that this parameter is only capable of expressing the total bacterial burden of a respiratory sample, but it is not useful to distinguish colonization from pneumonia.

In summary, the present study shows that EA quantitative cultures using a cut-off point between 10³ and 10⁶ *cfu/ml* may play a practical role in diagnosing some of the VA pneumonias. However, this technique cannot replace PSB or BAL, since these have been demonstrated to be more specific.

References


