Diagnostic Techniques and Procedures for Establishing the Microbial Etiology of Ventilator-Associated Pneumonia for Clinical Trials: The Pros for Quantitative Cultures

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Use of only clinical criteria for enrolling patients in clinical trials leads to inclusion of many patients who have no pulmonary infection, which can make the evaluation of new treatment modalities difficult. Quantitative cultures of specimens obtained using bronchoscopic or nonbronchoscopic techniques, such as bronchoalveolar lavage and/or protected specimen brush, are much more specific and could improve identification of patients with ventilator-associated pneumonia. Microscopic examination of distal respiratory secretions with use of Gram staining permits randomizing only patients with a high probability of ventilator-associated pneumonia and, thus, avoids the potential bias that can result from secondary exclusions. Invasive techniques also offer a sensitive and specific approach for identifying the responsible microorganisms, which is particularly important when evaluating antimicrobial agents for which bactericidal activity can be highly variable from one pathogen to another. Follow-up evaluation of the infected site with use of the same techniques permits determination of the pharmacokinetic and/or pharmacodynamic parameters of the new agents and their microbiological efficacy, compared with current antibiotics.

Concern about the inaccuracy of clinical approaches to distinguish patients receiving mechanical ventilation who have only proximal airway colonization from patients with true bacterial ventilator-associated pneumonia (VAP) has led numerous investigators to postulate that quantitative cultures of distal respiratory secretions obtained with bronchoscopic or nonbronchoscopic techniques, such as bronchoalveolar lavage (BAL) and/or protected specimen brush (PSB), could improve identification of patients with true VAP and, thus, facilitate the conduct of clinical trials in this context [1–4]. By using such techniques, inclusion criteria and therapeutic decisions can be tightly protocolized on the basis of results of direct examination of distal pulmonary samples and results of quantitative cultures, avoiding enrolling patients who have no pulmonary infection. This article reviews the potential advantages and drawbacks of using quantitative cultures of distal respiratory secretions, compared with noninvasive modalities or clinical evaluation for the diagnosis of VAP, based on our personal experience and recent, major additions to the literature.

POTENTIAL DRAWBACKS AND LIMITATIONS OF CLINICAL APPROACHES

The diagnosis of VAP is usually based on 3 components: systemic signs of infection, new or worsening infiltrates seen on the chest roentgenogram, and bacteriologic evidence of pulmonary infection [5–8]. Systemic signs of infection, such as fever, tachycardia, and leukocytosis, are nonspecific findings that can be caused by any condition that releases cytokines. Hemorrhage, soft-tissue trauma, and thermal injury are among the noninfec-
tious conditions that are associated with elevated circulating concentrations of cytokines. In trauma and other surgical patients, fever and leukocytosis should prompt the physician to suspect infection, but in the early posttraumatic or postoperative period (ie, during the first 72 h), these findings are not usually helpful. Later in the course, fever and leukocytosis are more likely to be caused by infection, but even at that time, other problems associated with an inflammatory response (eg, devitalized tissue and open wounds) can cause these findings.

The plain (usually portable) chest roentgenogram remains an important component in the evaluation of hospitalized patients with suspected pneumonia, although it is most helpful when the findings are normal and rule out pneumonia. When infiltrates are evident, the particular pattern is of limited value for differentiating among cardiogenic pulmonary edema, non-cardiogenic pulmonary edema, pulmonary contusion, atelectasis (or collapse), and pneumonia. In a review of 24 patients with autopsy-proven pneumonia who were receiving mechanical ventilation, no single radiographic sign had a diagnostic accuracy >68% [9]. The presence of air bronchograms was the only sign that correlated well with pneumonia, correctly predicting 64% of pneumonia cases in the entire group. However, in patients with acute respiratory distress syndrome, a variety of causes other than pneumonia can explain asymmetric consolidation (eg, atelectasis, emphysema, pulmonary edema, and/or thromboembolic disease). Marked asymmetry of radiographic abnormalities has also been reported in patients with uncomplicated acute respiratory distress syndrome.

Although tracheal secretions are easily obtainable by direct suctioning through the endotracheal tube, results of microscopic evaluation and culture of these samples are frequently inconclusive in patients with a clinical suspicion of VAP, because the upper respiratory tract of most intensive care unit patients is colonized with potential pulmonary pathogens when deep pulmonary infection is present and not present [10, 11]. Compared with culture of open lung biopsy samples or other specimens, such as those obtained through protected specimen brushing, culture analysis of tracheal aspirate specimens obtained from patients receiving mechanical ventilation showed moderate-to-high sensitivity but generally low specificity [1]. Hill et al [12] obtained simultaneous samples of the deep trachea and lung from 48 patients with respiratory failure through open lung biopsy. Culture results agreed in only 40% of these paired samples. For patients with pneumonia documented by histologic evidence, the sensitivity of culture of endotracheal aspirate specimens was 82%, but specificity was only 27% [12]. The potential usefulness of routine culture of endotracheal aspirate specimens for monitoring the response to antibiotic treatment in patients with VAP is also questionable, because the upper respiratory tract of most patients with pneumonia remains colonized with multiple potential pathogens, even when the clinical course is favorable. These cultures contribute indisputably to the diagnosis of VAP only when their results are completely negative for a patient with no modification of prior antimicrobial treatment. In such a case, the negative predictive value is very high, and the probability that the patient has pneumonia is close to nil [13]. These patients should not be included in clinical trials evaluating a new modality of treatment for VAP.

In 1991, Pugin et al [14] described a composite clinical score based on 7 variables (temperature, blood leukocyte count, volume and purulence of tracheal secretions, oxygenation, pulmonary radiograph findings at baseline and on days 2–3, and results of semiquantitative culture of tracheal aspirate specimens). In this study involving 28 patients requiring prolonged mechanical ventilation, a good correlation was observed between this clinical score and quantitative bacteriologic examination of BAL samples, with a cutoff of 6 enabling identification of patients with infection. However, subsequent studies have not confirmed the diagnostic accuracy of this score or its reproducibility [15–17]. One additional potential difficulty for using this score as an inclusion criterion in trials designed for evaluating a new treatment is that 2 of the 7 parameters mandatory for calculating the score are only available on days 2–3, namely, progression of pulmonary infiltrates and results of semiquantitative cultures of endotracheal aspirate specimens. Although the potential usefulness of this score for enrolling patients in a clinical trial is questionable, it can probably be used as an objective marker of outcome when the 5 parameters (body temperature, leukocyte count, tracheal secretions, factors, oxygenation ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen, and pulmonary radiograph findings) are determined serially during the follow-up period, in conjunction with parameters that are usually used for defining success and failure, such as lack of need for additional antibiotics and survival [18–20].

**POTENTIAL USEFULNESS OF QUANTITATIVE CULTURES OF DISTAL RESPIRATORY SECRETIONS**

BAL or PSB permits collection of distal pulmonary secretions with minimal or no upper airway contamination, either through a fiberoptic bronchoscope or blindly, using an endobronchial catheter that is wedged in the tracheobronchial tree [1, 11]. Because of the inevitable oropharyngeal bacterial contamination that occurs in the collection of all respiratory secretion samples, quantitative culture techniques are always needed to differentiate oropharyngeal contaminants present at low concentration from higher-concentration infecting organisms. Because even a few doses of a new antimicrobial agent can negate results of microbiologic cultures, pulmonary secre-
tions from patients suspected of having pneumonia should always be obtained before new antibiotics are administered [1].

Although some investigators have concluded that culture of BAL fluid specimens provides the best reflection of the bacterial burden in the lungs, both quantitatively and qualitatively, others have reported mixed results, with lower specificity of BAL fluid cultures for patients with high tracheobronchial colonization, compared with culture of PSB samples [21–24]. When the results of the 11 studies evaluating BAL fluid specimens from a total of 435 intensive care unit patients suspected of having nosocomial pneumonia were pooled, the overall accuracy of this technique was found to be very close to that of PSB; the Q value was 0.84 (Q represents the intersection between the summary receiver-operating characteristic [ROC] curve and a diagonal from the upper left corner to the lower right corner of the ROC space) [21]. Similar conclusions were drawn in another meta-analysis when the results of 23 studies were pooled [23].

Other studies have confirmed the accuracy of bronchoscopic techniques for diagnosing VAP. In a study evaluating spontaneous lung infections occurring in baboons with permeability pulmonary edema and undergoing mechanical ventilation, Johanson et al [25] found an excellent correlation between the bacterial content in lung tissue and results of quantitative culture of BAL fluid and PSB specimens. Culture of BAL fluid specimens recovered 74% of all species present in lung tissue, including 100% of those present at a concentration ≥1 × 10⁴ culture-forming units (cfu)/g of tissue. In this study, culture of PSB specimens identified only 41% of all species recovered from lung tissue; however, of note, only microorganisms present at very low concentrations in the lung were missed, because 78% of species present at concentrations ≥1 × 10⁴ cfu/g of tissue were isolated correctly. Similarly, in a study involving 20 patients receiving mechanical ventilation who had not developed pneumonia before the terminal phase of their disease and who had no recent changes in antimicrobial therapy, Chastre et al [26] found that cultures of bronchoscopic PSB and BAL fluid specimens obtained just after death were able to identify 80% of all species present in the lung, with a strong correlation between the results of quantitative cultures of both specimens and lung tissue. Despite the need for cautious interpretation, the results of those studies indicate that invasive techniques offer a sensitive and specific approach to differentiate between colonization of the upper respiratory tract and distal lung infection and to identify the microorganisms involved in patients with VAP. Use of these techniques could therefore facilitate a per-pathogen analysis when evaluating a new antimicrobial agent for which bactericidal activity is different from one pathogen to another.

Because BAL harvests cells and secretions from a large area of the lung that can be microscopically examined immediately after the procedure to detect the presence or absence of intracellular or extracellular bacteria in the lower respiratory tract, it is particularly well suited to provide rapid identification of patients with VAP [26]. In a study in which the diagnostic accuracy of direct microscopic examination of BAL cells could be assessed directly with both histologic and microbiologic postmortem lung features in the same segment, a very high correlation was shown between the percentage of BAL cells containing intracellular bacteria and the total number of bacteria recovered from the corresponding lung samples and with the histologic grades of pneumonia [26]. Several other studies have confirmed the diagnostic value of this approach [26–31]. Use of the presence of bacteria on direct examination of BAL cells and fluid as an inclusion criterion would be a major advantage in the conduct of clinical trials, because it permits randomization of only patients with a high probability of VAP and, thus, avoids secondary exclusions and the potential bias that they represent in the intent-to-treat analysis of randomized trials.

Finally, follow-up evaluation of the infected site in the lung with use of the same techniques may permit determination of the pharmacokinetic and/or pharmacodynamic parameters of antimicrobial agents and their microbiological efficacy. Several published reports have revealed a relationship among serum concentrations of β-lactams or other antibiotics, the minimum inhibitory concentration of the infecting organism, and the rate of bacterial eradication from respiratory secretions in patients with lung infection [32, 33]. Consequently, determination of time to pathogen eradication could represent a valid end point in clinical trials comparing 2 antibiotics, leading to a more precise evaluation of their potential efficacy.

**POTENTIAL LIMITATIONS OF QUANTITATIVE TECHNIQUES**

Four studies using a protocol based on postmortem lung biopsies have suggested that, in the presence of prior antibiotic treatment, many patients with histopathologic signs of pneumonia have no or only minimal growth on bronchoscopic specimens cultures, casting some doubt on the ability of invasive techniques for diagnosing VAP [13, 34–36]. For example, in a study involving 30 patients who died during receipt of mechanical ventilation after having received prior antibiotic treatment, Torres et al [37] found that quantitative bacterial cultures of PSB and BAL fluid specimens had low sensitivity (36% and 50%, respectively) and low specificity (50% and 45%, respectively) and could not differentiate between the histologic absence or presence of pneumonia.

Unfortunately, several methodological constraints specific to the evaluation of procedures used in the diagnosis of bacterial pneumonia were not respected in these studies [13, 34–37]. None of them excluded patients who had received new anti-
bacteriostats in the days preceding death or patients who had developed a first episode of either community-acquired or nosocomially acquired pneumonia. These limitations could explain why, in those studies, quantitative bacterial cultures of lung biopsy specimens had no better predictive utility than did bronchoscopic techniques. Diagnostic methods based on microbiologic techniques can only document, both qualitatively and quantitatively, the bacterial burden present in lung tissue. In any of these instances, these techniques cannot identify resolving pneumonia in retrospect, at a time when antimicrobial treatment and lung antibacterial defenses might have been successful in suppressing microbial growth in lung tissue. Use of histologic criteria as a reference implies that the patient had not developed a lung infection many days before the episode under evaluation; otherwise, it would be difficult (if not impossible) to distinguish a recent infection from the sequelae of a previous infection and, thus, to correctly interpret the results of the diagnostic tool(s) under evaluation. Of interest, when analyses in these 4 studies were re-analyzed to patients with no prior antibiotic therapy or when only lung tissue cultures were used as the gold standard, results obtained using bronchoscopic techniques for diagnosing VAP were much better, with a sensitivity always >80%.

As indicated above, the accuracy of bronchoscopic techniques is questionable in patients receiving antibiotic therapy, especially when new antibiotics have been introduced after the onset of the symptoms suggestive of VAP, before pulmonary secretions are collected. In fact, as demonstrated by several investigators, results of culture of respiratory secretions are mostly not modified when pneumonia develops as a superinfection in patients who have been receiving systemic antibiotics for several days before the appearance of the new pulmonary infiltrates, because the bacteria responsible for the new infection are then resistant to the antibiotics given previously [25, 38]. On the other hand, performance of microbiologic cultures of pulmonary secretions for diagnostic purposes after initiation of new antibiotic therapy in patients suspected of having developed VAP can clearly lead to a high number of false-negative results, regardless of the way in which these secretions are obtained [38–40].

Although a general consensus has emerged on the use of $1 \times 10^3$ cfu/mL as the cutoff for culture of PSB specimens and $1 \times 10^4$ cfu/mL for culture of BAL fluid specimens, concern has been raised about the reproducibility of these results, particularly near the diagnostic thresholds. Three groups [41–43] have concluded that, although in vitro repeatability is excellent and in vivo qualitative recovery is 100%, quantitative results are more variable. For 14%–17% of patients, results of culture of replicate samples decreased on both sides of the $1 \times 10^3$ cfu/mL threshold, and results varied by >1 log10 for 59%–67% of samples. Finally, many microbiology laboratories may not be able to promptly and accurately process quantitative cultures, although the techniques used can be very similar to those applied routinely to urine cultures.

Several investigators also argue that requiring the use of bronchoscopy for documenting VAP in clinical trials may render the conduct of these trials difficult, because only a limited number of centers are using these techniques routinely. However, nonbronchoscopic methods using quantitative cultures may be an acceptable tool for diagnosing VAP when no fiberoptic techniques are available. At least 15 studies have described a variety of nonbronchoscopic techniques for obtaining samples of lower respiratory tract secretions; results have been essentially similar to those obtained in studies using more-invasive techniques, even if some patients with VAP may be missed by these techniques because of the potential sampling errors inherent in a blind technique and the lack of airway visualization [44, 45]. Advantages of these techniques are less invasiveness, availability to clinicians not qualified to perform bronchoscopy, and ease of performance 24 h per day every day, even in patients intubated with small endotracheal tubes. Use of these techniques by clinical centers without the capability to routinely perform fiberoptic bronchoscopy may facilitate the conduct of clinical trials.

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