TECHNICAL NOTE

Ventilator associated pneumonia: quality of nonbronchoscopic bronchoalveolar lavage sample affects diagnostic yield

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Ventilator associated pneumonia: quality of nonbronchoscopic bronchoalveolar lavage sample affects diagnostic yield. R.P. Baughman, R.E. Spencer, B.O. Kleykamp, M.C. Rashkin, M.M. Douthit. ©ERS Journals Ltd 2000.

ABSTRACT: The importance of predefined criteria for acceptable samples of respiratory therapists' obtained lower respiratory samples were studied, using a nonbronchoscopic bronchoalveolar lavage (BAL) protocol for ventilated patients in the intensive care unit.

Therapists were instructed and asked to follow guidelines for obtaining samples. Over one year, 219 samples were obtained by respiratory therapists. Of these, 115 were considered to be adequate samples using the following criteria: 60 mL of instilled volume, at least 5 mL of fluid aspirated, specimens sent for semiquantitative culture, a differential cell count of <5% bronchial epithelial cells.

Overall, 52 samples grew one or more pathogen at >10,000 colony forming units (cfu)·mL⁻¹ of BAL. The most common pathogen was *Staphylococcus aureus* (*S. aureus*) (11 samples), although Gram-negative bacilli were the single pathogen in 21 specimens. Of the 115 acceptable samples, 40 (35%) grew ≥1 pathogen at >10,000 cfu·mL⁻¹. For the 80 not acceptable samples which were sent for appropriate culture, 12 (15%) grew >10,000 cfu·mL⁻¹ BAL. This difference was significant (Chi-squared=9.44, p<0.01).

Nonbronchoscopic bronchoalveolar lavage can be safely performed by respiratory therapists'. The authors recommend that a protocol be used to evaluate the quality of a bronchoalveolar lavage sample in the same manner sputum samples are screened prior to interpretation.

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The diagnosis of ventilator associated pneumonia (VAP) remains a major clinical problem [1, 2]. Over the past 10 years, multiple invasive and noninvasive techniques have been studied in order to improve the diagnostic sensitivity and specificity [3–7]. Clinical criteria alone are insufficient, with up to half of the patients with clinical criteria for pneumonia having some other diagnosis [8, 9]. Invasive diagnostic procedures include protected brush specimens (PBS) and bronchoalveolar lavage (BAL). These techniques require use of semiquantitative cultures [10, 11], and allow for the measurement of bacterial infectious load as well as proving to be fairly specific in diagnosing lower respiratory infection. Unfortunately, however, the yield is low. Among the factors which appear to worsen the yield is the limited access to bronchoscopy, especially whenever antibiotics are changed.

The introduction of nonbronchoscopic BAL has offered the ability to obtain lower respiratory samples without using a bronchoscope [3, 4, 12]. Using semiquantitative cultures, the results of nonbronchoscopic BAL have been comparable to invasive diagnostic procedures. In a study comparing the results of immediate post mortem lung samples *versus* nonbronchoscopic BAL samples, the technique has proved both sensitive and specific [13, 14].

A major advantage of nonbronchoscopic BAL is that it can be performed by nonphysicians, especially respiratory

therapists, who are available for 24 h in most intensive care units [15]. However, some way of assessing the adequacy of the sample taken by respiratory therapists' may be required. Criteria for adequate sampling have not been established. In the present study criteria were established for the performance of nonbronchoscopic BAL by respiratory therapists'. The results of the compliance with the protocol and its effect on diagnostic yield in patients with possible VAP are reported.

Methods

Patients seen in the Cincinnati Medical Intensive Care Unit, over a one-year period, were eligible for the study. Patients with possible pneumonia were enrolled. Criteria for pneumonia included ≥2 of the following: increased pulmonary secretions, fever, leukocytosis or leukopenia, or worsening hypoxia. All patients with new or worsening infiltrates by chest roentgenogram were evaluated for the nonbronchoscopic BAL protocol. Patients whose partial pressure of oxygen (PO₂) was <60 mmHg on 100% oxygen or who had a coagulopathy (platelets <50,000·mm⁻³) were excluded from the study.

For the nonbronchoscopic BAL protocol, the patient was initially placed on 100% oxygen. The BAL catheter

(Ballard, Draper, Utah, USA) was introduced through a special adapter placed at the end of the endotracheal tube [12]. The outer catheter is marked with a black line ~30 cm from the tip of the catheter. The catheter was passed until this black line lined up with either the lip (for an endotracheal tube) or nares (for a nasotracheal tube). The inner catheter was advanced distally until it met resistance (wedged). Two aliquots of 30 mL of normal saline were introduced and aspirated using a hand held syringe.

The respiratory therapists underwent training sessions. They were asked to fill in a form for each nonbronchoscopic BAL with the volume instilled and returned. Aliquots were to be sent for semiquantitative culture [10]. Another aliquot was sent for cell count and cytocentrifuge prepared slides. The slides were stained using a modified Wright Giemsa stain, and a differential cell count was performed on 200 nucleated cells.

Comparisons were made using Chi-squared analysis. The sensitivity, specificity, positive and negative predictive value, and likelihood ratio were calculated using standard formulas.

Results

A total of 219 nonbronchoscopic BAL studies were performed over a one-year period. For the sample to be considered adequate, the following criteria were established: 1) the instilled volume must be 60 mL; 2) the aspirated volume needed to be at least 5 mL; 3) specimens needed to be sent for semiquantitative culture; 4) a differential cell count of <5% epithelial cells. Table 1 summarizes the break down of the samples, including the reason for excluding them as acceptable samples. The most common protocol violations dealt with volume of fluid, with 20 cases not having a sample recorded and another 55 samples having an instilled volume other than 60 mL. A total of 24 samples were not sent for semiquantitative cultures. Interestingly, of the samples using 60 mL instilled and at least 5 mL aspirated volume, only five had more than 5% bronchial epithelial cells. The final number of nonbronchoscopic BAL samples which followed all criteria was 115 (53% of total sent). These were called the acceptable nonbronchoscopic BAL samples and were compared to the 80 samples which were sent for culture, but considered not acceptable based on one or more criteria. There were 24 samples which were not analysed since they were not sent for culture.

The number of nonbronchoscopic BALs performed over time were analysed. There was a seasonal variation, with the majority of procedures carried out in the winter months, reflecting the increased incidence of pneumonia seen

Table 1. – Evaluation of samples and reason for exclusion

Criteria	Samples meeting criteria n	Samples failing criteria n
Total samples sent	219	–
Volume recorded	199	20
60 mL saline instilled	144	55
At least 5 mL aspirated	144	0
Sent for semi-quantitative culture	120	24
<5% bronchial epithelial cells	115	5

Fig. 1. – Results of the semiquantitative cultures for all isolates. The isolates which were >10,000 cfu·mL⁻¹ bronchoalveolar lavage (BAL) were subsequently compared to those <10,000 cfu·mL⁻¹ BAL. cfu: colony forming units. □: Pneumonia; ▨: not pneumonia.

during that time. The percentage of samples performed correctly improved over time. However, some samples were not acceptable even in the last months of the study (data not shown).

The culture results are summarized in figure 1. There was a biphasic distribution, with a drop at 1,000 cfu·mL⁻¹. The use of >10,000 cfu·mL⁻¹ as the criteria for significant pathogens has proved to be a useful cut-off between pneumonia and not pneumonia in other studies [10, 16] and represents the recommended cut-off for bronchoscopic BAL [11]. It has also been verified for nonbronchoscopic BAL [12, 17]. Patients with >10,000 cfu·mL⁻¹ of BAL fluid were diagnosed as having pneumonia. Table 2, summarizes the bacteria recovered for the patients with both acceptable samples as well as the whole group. The most common class of organisms overall was Gram negative organisms. The most commonly recovered single organism was *S. aureus*.

The value of using a protocol for performing and assessing the nonbronchoscopic BAL results was then examined. The diagnostic yield for bacteria >10,000 cfu·mL⁻¹ BAL fluid from two groups was compared, the samples in which the protocol was followed and sample was adequate (acceptable) versus those in whom cultures were sent but the protocol was not followed (not

Table 2. – Organisms recovered at >10,000 cfu·mL⁻¹ in bronchoalveolar lavage

Organism	Acceptable samples	All samples
<i>Pseudomonas aeruginosa</i>	5	9
<i>Escherichia coli</i>	1	1
<i>Klebsiella pneumoniae</i>	2	2
<i>Haemophilus influenzae</i>	5	6
<i>Pseudomonas alcaligenes</i>	1	2
<i>Enterobacter</i>	1	1
<i>Staphylococcus aureus</i>	9	11
<i>Streptococcus pneumoniae</i>	3	4
<i>Enterococcus</i>	3	3
Mixed	10	13
Total	40	52

Fig. 2. – Comparison of the number of samples with $>10,000$ cfu·mL⁻¹ bronchoalveolar lavage for the acceptable *versus* not acceptable samples. The rate was significantly higher in the acceptable group (Chi-squared=8.06, $p<0.01$). ▨: $>10,000$ colony forming units·mL⁻¹; □: $<10,000$ colony forming units·mL⁻¹.

acceptable). The most common reason for an unacceptable sample was using an incorrect volume. The volumes which were recorded but in violation of protocol included 29 samples where <60 mL was introduced and 26 cases where >60 mL was introduced. In the larger volume samples, the usual reason was that insufficient fluid was aspirated after the first 60 mL was introduced. In 20 cases, it was not known how much fluid was introduced. For the 115 acceptable samples 40 (35%) were $>10,000$ cfu·mL⁻¹ of BAL, while only 12 of the 80 (15%) of the not acceptable samples had $>10,000$ cfu·mL⁻¹ of BAL bacteria recovered (fig. 2). This difference was significant (Chi-squared=9.44, $p<0.01$).

Figure 3 gives a breakdown of the results of the 115 nonbronchoscopic BAL samples where 60 mL was introduced, the samples were sent for semiquantitative culture, there was $<5\%$ bronchial epithelial cells, and at least 5 mL of aspirated fluid. In all 115 cases, there were ≥ 6 mL of fluid aspirated. There was no difference in the percentage of positive samples for those patients in whom a larger volume of fluid was aspirated than if 5 mL was used as the acceptable minimal volume. Over a third of the samples had 5–10 mL of aspirated fluid.

Fig. 3. – Volume of aspirated fluid for acceptable samples. All had 60 mL fluid instilled. The number of specimens with $>10,000$ cfu·mL⁻¹ (▨) bronchoalveolar lavage (BAL) and $<10,000$ cfu·mL⁻¹ (□) BAL for different volumes of aspirated fluid are shown. The proportion of samples with positive cultures were no different for the different volumes of aspirated fluid.

Fig. 4. – The cellular differential count of the bronchoalveolar lavage (BAL) fluid samples for patients with acceptable samples. The groups with $>10,000$ cfu·mL⁻¹ BAL had a higher percentage of neutrophils than those with $<10,000$ cfu·mL⁻¹ BAL. However, there was considerable overlap. cfu: colony forming units.

The inflammatory response of the lung is one method to detect pneumonia. In examining the patients with acceptable samples, the diagnostic value of the BAL differential counts were examined. Since neutrophils in the BAL would be expected in pneumonia, the differential counts for those with and without $>10,000$ cfu·mL⁻¹ BAL were compared. Figure 4 summarizes the percentage of neutrophils found in the BAL for patients with $>10,000$ cfu·mL⁻¹ BAL *versus* those with $<10,000$ cfu·mL⁻¹ BAL. There was a significant difference between the per cent neutrophils for those with $>10,000$ cfu·mL⁻¹ BAL (median (range): 74 (2–97%)) *versus* those with $<10,000$ cfu·mL⁻¹ BAL (11 (0–97%)), $p<0.0001$). Normal healthy nonintubated controls undergoing routine BAL have $<2\%$ neutrophils in their BAL. There was no difference in the per cent lymphocytes ($>10,000$ cfu·mL⁻¹ BAL 1 (0–10%); $<10,000$ cfu·mL⁻¹ BAL 2 (0–21%)) or eosinophils ($>10,000$ cfu·mL⁻¹ BAL 0 (0–4%); $<10,000$ cfu·mL⁻¹ BAL 0 (0–5%)). Overall, only 2 of the 40 with $>10,000$ cfu·mL⁻¹ BAL had $<20\%$ neutrophils while 41 of 66 (65%) of those with $<10,000$ cfu·mL⁻¹ BAL had $<20\%$ neutrophils in their BAL (Chi-squared=35.24, $p<0.001$). The cut-off of 20% neutrophils had a sensitivity of 95%, specificity of 62%, a positive predictive value of 62%, and a negative predictive value of 95%. The likelihood ratio of a patient with $>20\%$ neutrophils in their BAL having $>10,000$ cfu·mL⁻¹ BAL was 2.5.

The respiratory therapist also provided information regarding complications from the procedure. During the time of the study, only two patients were reported to have persistent hypoxaemia (saturation $<90\%$) on 100% oxygen for >30 min after the procedure. Most patients were quickly weaned back to the level of oxygen prior to the procedure.

Discussion

This study demonstrated that sampling of lower respiratory secretions can be obtained using a nonbronchoscopic BAL procedure. This technique can be performed by nonphysician personnel who can follow a regular protocol. The use of pre-established criteria for the acceptable

sample could reduce the number of procedures to be analysed. However, the overall yield using this nonbronchoscopic BAL for these acceptable samples was >35%.

Several studies have examined the methods of diagnosing VAP. The use of clinical criteria alone have been supported by several observations [1]. An important factor is that the use of antibiotics guided by BAL results have not changed clinical outcome [18]. The study by LUNA *et al.* [18] demonstrated that the most important feature affecting prognosis was the ability to start the correct empiric antibiotics, also noted by others [15]. If the initial empiric therapy did not cover all the significant isolates in the BAL, then switching antibiotics to cover the antibiotic resistant organism appeared to have little effect on mortality. However, it could be argued that knowing what the current flora is in an individual intensive care unit (ICU) is important for choosing the correct empiric antibiotics [19]. The proponents of diagnostic sampling have pointed out that clinical criteria alone misidentify up to half of the patients with possible VAP [3, 7, 20]. This includes the overuse of antibiotics for pathogens colonizing the upper airways. Another major problem is the physician missing other treatable problems, such as congestive heart failure, pulmonary emboli, or sinusitis [21].

In a prior study carried out in the authors ICU, 173 bronchoscopic lavages were performed for possible VAP, and only 23 (13%) of the lavage samples grew >10,000 cfu·mL⁻¹ of BAL [22]. Major drawbacks for the bronchoscopic BAL sample have been the cost and difficulty in timing the samples. It appears that the yield would be higher if samples were obtained at the time of the change of antibiotic, rather than waiting until a bronchoscopy can be performed. Although some studies have failed to demonstrate an effect of antibiotics on the yield of bronchoscopic BAL [3], most clinicians would agree that the earlier the sample, the better the results. The mechanism for increasing early samples was to train the respiratory therapists to perform the procedure, this has been shown to improve yields by other studies [4, 15]. However, those authors did not appear to have criteria for an acceptable sample. For bronchoscopic BAL, it is known that not all lavage samples are adequate. Therefore, it was felt that nonbronchoscopic BAL would also require some criteria for acceptable sample.

One of the difficulties with BAL over the years has been the lack of a standard technique. Many different ways to perform and interpret BAL have been published. Although guidelines for performing BAL and reporting the results have been published [23–25], these have usually been ignored. This has been in part because of the variability inherent in the lavage process itself [24]. Also, for some pneumonias, the technique is not that important. For a patient with malignancy or *Pneumocystis carinii* (*P. carinii*), the sample is always interpretable [26]. In patients with *P. carinii*, small volume lavages have as high a rate of diagnosis as larger volume lavages [27, 28]. However, it is clear that smaller volumes are associated with a larger bronchial component. In comparing the yield from the first 20 mL of introduced fluid to the remaining fluid, RENNARD *et al.* [29] found a higher percentage of epithelial cells and a lower percentage of alveolar macrophages. Further studies by that group found higher levels of lactoferrin, a protein made by

bronchial cells [30]. An increase in the percentage of bronchial cells in the first aliquot was also noted, as well as an increase in the proportion of immunoglobulins A and G, again indicating that this small volume lavage was associated with bronchial sampling [31].

The use of criteria to assess samples has been carried out for years with sputum [32]. The present study proposes criteria for acceptable nonbronchoscopic BAL samples. The finding of neutrophils in the BAL fluid of the patients was not helpful in assessing samples. There was considerable overlap between the infected and noninfected patients. Several conditions lead to increased neutrophils without infection necessarily being present, including acute respiratory distress syndrome [33, 34]. Increased neutrophils are also seen as the result of intubation and ventilation itself [35].

It has been suggested that the percentage of epithelial cells be used as a marker of bronchial contamination [23]. Samples with >5% epithelial cells should not be considered a true alveolar sample. Some groups have used cytologic evaluation in assessing their sample [14, 17, 36]. The widespread use of this guideline has been recommended when using invasive techniques to diagnose VAP [37]. In addition, the minimally acceptable volume of return is not clear. A recommendation has been made that a minimum volume of return be specified; for BAL, that minimum appears to be 10% of the instilled volume [38]. In this study, 5 mL was chosen, representing ~10% of the instilled volume. Using a higher volume of return was associated with a much smaller number of acceptable samples and no difference in the per cent of samples with >10,000 cfu·mL⁻¹.

The use of standards for an acceptable *versus* nonacceptable sample led to a significantly higher yield for the acceptable sample. This would support the concept that deeper specimens would be more reliable than upper airway secretions. The use of semiquantitative cultures of tracheal aspirates was as good as deeper samples in one pilot study [39], the group had previously shown that the endotracheal aspirate was not as specific as deeper samples [40]. Therefore, their pilot study may not adequately answer the question of whether invasive specimens are necessary. Others have found very weak correlation between culture results of endotracheal aspirates and bronchoscopic obtained samples [41]. The present study did not systematically obtain tracheal aspirate samples, so the culture results of this study could not be compared to tracheal culture results.

The goal of this study was to determine whether deep respiratory samples could be reliably obtained. It was found that the respiratory therapists' could be trained to carry out this procedure. The risk of the study was low, with only two patients having persistent hypoxaemia. However, one requirement was that all patients be adequately oxygenated at the time of the procedure, with a PO_2 of >60 mmHg with supplemental oxygen, since this group of patients may develop significant hypoxaemia associated with the lavage process [6]. The authors found that criteria for an adequate sample could rely on only a few pieces of information. Therefore, a physician did not have to be immediately available to assess the procedure or sample. Thus, the procedure could be performed "off hours", especially prior to changing antibiotics. This may increase the acceptance of the procedure.

To conclude, nonbronchoscopic bronchoalveolar lavage can be performed safely by respiratory therapists'. Establishing criteria for an adequate sample allows for an assessment of the reliability of the results of the sample. An acceptable sample was associated with a higher proportion of cultures $>10,000$ cfu·mL⁻¹ of bronchoalveolar lavage in patients with suspected ventilator associated pneumonia.

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