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## CHAPTER 17

## Bronchoalveolar Lavage

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*Background and Definition*

Bronchoalveolar lavage (BAL) is a diagnostic technique for sampling from the alveoli and small airways. It is also helpful in the diagnostic evaluation of some interstitial diseases.<sup>1-3</sup> Sterile isotonic saline is instilled into a bronchus in a volume large enough to reach the alveoli communicating with the airway. The saline is then retrieved for analysis, along with cells and other compounds lining the airways and alveoli. Bronchoalveolar lavage is most commonly performed during bronchoscopy. Fortunately, simple, inexpensive techniques that can be performed in any practice have recently been described for nonbronchoscopic BAL.

Bronchoalveolar lavage is distinct from tracheal or bronchial washes. Airway washes collect material from the surface of large airways only. Bronchoalveolar lavage collects material from deep within the lung, with only minor contribution from the large airways except in cases of bronchitis. The retrieval of material from the deep lung by BAL is identifiable by a grossly visible layer of foam on top of the fluid indicating the presence of surfactant and cytologically (in health) by large numbers of alveolar macrophages within the fluid.

Many veterinarians and veterinary students are initially dismayed by the relatively large volumes of saline instilled into the lungs to perform BAL; therefore the technique is still not used routinely in nonreferral settings. However, BAL has been used widely for many years in people and animals of all sizes, and the effects of BAL have been studied and reported in detail. In the 1970s, using healthy dogs to study the possibility of therapeutic whole lung lavage with saline, effects were transient even using vol-

umes of saline as high as 4 liters/dog.<sup>4-7</sup> Bronchoalveolar lavage is considered a routine diagnostic technique in human medicine and should be employed routinely in the diagnosis of certain lung diseases in veterinary medicine as well. The ability to perform BAL sequentially in the same individual also makes it a powerful tool in the investigation of disease progression or therapeutic response.

A sufficient volume of BAL fluid can be recovered for most types of analysis. Routine analysis of fluid in the veterinary clinical setting consists of cytology, bacterial culture, and sometimes fungal or mycoplasmal culture. Other diagnostic assays that are used in the clinical analysis of BAL fluid from human patients include viral cultures, antigen tests for cryptococcosis, polymerase chain reaction tests for specific organisms, and the application of monoclonal markers for specific tumors.

Bronchoalveolar lavage fluid analysis has also been used extensively in research settings to study local immune responses, cellular damage, and drug disposition in people and laboratory animals. Numerous papers have been published using dogs and cats as subjects. For instance, immune responses have been investigated through cell counts, measurement of inflammatory mediators, cellular function assays, and maintenance and testing of macrophages in cell culture.<sup>8-17</sup> Phenotypic subtyping of lymphocytes in BAL fluid from healthy dogs and dogs undergoing treatment for pulmonary neoplasia has been reported.<sup>8,18,19</sup> Biochemical markers such as alkaline phosphatase and lactate dehydrogenase can be used as indicators of cellular damage.<sup>20,21</sup> In addition, studies of various drugs have used BAL as a means to estimate concentrations within the epithelial lining fluid (ELF) or phagocytes.<sup>22-24</sup>

There is one major technical difficulty in measuring components of BAL fluid. The degree of dilution imposed on the ELF is variable, depending on factors such as the volume of saline instilled, the volume of fluid recovered, and the dwell time of saline within the lung. This problem is rarely significant in the clinical situation because relative cell counts and the presence of pathogenic organisms or neoplastic cells are generally unaffected by the degree of dilution. Nevertheless, consistency in technique is helpful in the clinical setting and essential in research. Markers of dilution are often used in research to estimate the dilution of ELF. Urea and albumin are most commonly used, with urea being preferable in diseased populations.<sup>25</sup> Urea is freely diffusible and should be present in ELF at the same concentration as in plasma. If lavage is performed quickly, so that minimal additional urea can diffuse into the instilled saline, the volume of ELF in the BAL fluid can be calculated by the following formula<sup>26</sup>:

$$\text{Volume}_{\text{ELF}} = \text{Volume}_{\text{BAL}} \times \frac{\text{Urea}_{\text{BAL}}}{\text{Urea}_{\text{plasma}}}$$

This method is known to overestimate ELF volume, but with dwell times of less than 2 minutes the error is less than twofold.<sup>27,28</sup> No technique is entirely accurate in determining the volume of ELF in BAL fluid, and differences between study groups must be several-fold in order to be significant.

## Indications

Bronchoalveolar lavage is a valuable diagnostic technique for patients that are not in respiratory distress with lung disease involving the small airways, alveoli, or interstitium. Bronchoalveolar lavage should be performed routinely in patients undergoing diagnostic bronchoscopy because the additional risk is minimal. A large volume of lung is sampled by BAL, especially compared with lung aspiration. Large volumes of fluid are retrieved, providing abundant material for analysis. Note that tracheal wash usually provides an adequate specimen from patients with historic and radiographic findings suggestive of overt bacterial bronchopneumonia or aspiration pneumonia, and does not require the patient to undergo general anesthesia.

Bronchoalveolar lavage performed during bronchoscopy (B-BAL) can be directed to specific areas of the lung that are identified as abnormal by thoracic radiography or gross examination of the airways. B-BAL is therefore indicated in the investigation of localized disease or where gross examination of the airways or other bronchoscopic collection techniques will be useful. Nonbronchoscopic BAL (NB-BAL) does not allow for directed sampling, although likely collection sites can be presumed. NB-BAL is indicated in the investigation of diffuse lung disease when the equipment or expertise needed for bronchoscopy is not available, or when the owner's finances preclude bronchoscopy.

Bronchoalveolar lavage is used as a therapeutic modality in people with alveolar proteinosis, an uncommon

condition in which surfactant accumulates within the alveoli and interferes with ventilation. Lavage with many liters of saline is required for each treatment. A recent report describes treatment of a dog with alveolar proteinosis using lung lavage.<sup>29</sup> To the author's knowledge only one other dog and no cats have been reported to have this disease.<sup>30</sup> Bronchoalveolar lavage is not indicated in the treatment of aspiration pneumonia because it probably exacerbates airway obstruction by pushing particles deeper into the lung. At this time, BAL is considered primarily a diagnostic procedure in veterinary medicine.

## Diagnostic Yield

Techniques for collection of pulmonary specimens for cytological analysis, including BAL, offer the clear advantages of lower risk and less expense compared with lung biopsy, which is the gold standard for diagnosis of pulmonary disease. For any cytological specimen to provide an accurate diagnosis, the disease process must involve the specific area sampled; the diseased area must release organisms or abnormal cells into the collected material; and secondary infection, inflammation, or hemorrhage must not mask an underlying disease. Therefore, the diagnostic yield of any pulmonary specimen collection technique depends on patient selection and final diagnosis. As examples, tracheal wash has a high yield in the diagnosis of *Bordetella*-induced bronchitis but a low yield in the diagnosis of interstitial lung disease, and lung aspiration has a high yield in the diagnosis of neoplasia when the needle can be placed directly into a mass lesion.

The large volume of fluid retrieved for analysis by BAL increases its potential for providing a diagnosis, particularly compared with lung aspirates. Sufficient material is obtained for making multiple slides for special staining, for aerobic and anaerobic bacterial culture, for fungal or mycoplasmal culture, or for other specific tests (e.g., antigen assays or polymerase chain reaction [PCR]) in the investigation of infectious disease. The high quality cytological preparations that can be produced and large numbers of cells available for examination facilitate the diagnosis of neoplasia.

Several reports are available in the literature describing the diagnostic yield of BAL in dogs and cats. Unfortunately, total case numbers are low. A study of dogs with overt fungal pneumonia showed that organisms were detected cytologically in BAL fluid from 6 of 9 dogs (67%).<sup>31</sup> Tracheal wash of the same dogs resulted in diagnostic organisms in half of the dogs. *Cryptococcosis* was identified in BAL fluid from a cat with normal thoracic radiographs.<sup>32</sup> A study of dogs with multicentric lymphoma determined that identification of lung involvement by BAL exceeded the sensitivity of thoracic radiographs and was equal to that of histopathology based on previous reports.<sup>33</sup> Lymphoma was identified in 31 of 47 dogs (66%) by BAL and in only 16 dogs (34%) by radiography.<sup>33</sup> Tracheal wash was successful in identifying lymphoma in only 4 of 41 dogs (10%).<sup>33</sup> *Toxoplasma gondii* tachyzoites were found in BAL fluid from cats with experimentally induced infection in all cats with clinical signs and in many

without.<sup>34</sup> Lavage was more sensitive than histopathology in detecting organisms. Tachyzoites have also been found in BAL fluid from a client-owned cat with pneumonia due to toxoplasmosis.<sup>35</sup>

A retrospective study of dogs that underwent BAL at teaching hospitals found that BAL fluid cytology provided a definitive diagnosis in 17 of 68 cases (25%) and was supportive of the diagnosis in 34 cases (50%).<sup>36</sup> As with any cytological specimen, a definitive diagnosis was only possible when cells showed clear criteria of malignancy in the absence of inflammation or when intracellular bacteria or extracellular pathogens were seen. These numbers may underestimate the yield of BAL in routine practice because of the referral nature of the population. Referred cases may have failed diagnosis by other means and may have failed to respond to therapeutic trials for common diseases. On the other hand, only cases with a definitive clinical diagnosis were included in the analysis, which excluded cases in which BAL also failed to provide a diagnosis.

Information from the above studies regarding the diagnosis of neoplasia is useful.<sup>33,36</sup> Lymphoma of the lung is readily diagnosed by BAL. The technique should be considered for clinical trials where accurate staging of disease is needed and for treating patients that develop clinical or radiographic signs of lung disease to differentiate recurrence of neoplasia from infection. Carcinoma is much more likely to be identified than sarcoma. Carcinoma was definitively identified by BAL in 8 of 14 dogs (57%) and cells suspicious for malignancy were identified in an additional 4 dogs (29%), whereas sarcoma was identified in none of 7 dogs (0%).<sup>36</sup>

The value of BAL, as with all other pulmonary specimen collection techniques, is in positive findings. Identification of infectious agents or neoplastic cells can provide a definitive diagnosis. Characterization of the inflammatory response can be supportive of certain diagnoses, but failure to identify infectious agents or abnormal cells cannot be used to rule out specific diagnoses. The safety and low cost of BAL relative to lung biopsy makes attempts to obtain a positive result with this technique worthwhile.

## Side Effects

The physiological effects of BAL have been studied in detail in healthy subjects of many species. In humans, BAL is considered a safe procedure with minor side effects.<sup>3</sup> Effects on lung function include a transient decrease of forced expiratory volume in 1 second (FEV<sub>1</sub>), vital capacity (VC), peak expiratory flow (PEF), and arterial oxygen tension (PaO<sub>2</sub>). Bronchospasm is rare except in patients with hyperreactive airways. Fever is seen in 10% to 30% of patients and usually responds to antipyretics.

In dogs the effects of whole lung lavage using much larger volumes of saline than those used for diagnostic purposes have been described.<sup>4-6</sup> These studies were performed to investigate the feasibility of repeated, large volume, whole lung lavages to remove inhaled radioactive particles accumulating in the lungs following a nuclear

accident. Early alterations include decreased arterial oxygen tension; increased respiratory rate; and smaller tidal volumes, which appear to be the result of ventilation/perfusion abnormalities due to retention of saline and loss of surfactant. Some fluid is retained within the lungs following lavage, but within 48 hours there were minimal histologic changes or pulmonary function abnormalities. No cumulative or long-term effects were reported. Further, diagnostic lavage can be repeated at 48-hour intervals without affecting the cytological findings within the fluid.<sup>7</sup> Body temperatures over 39.5° C were found following BAL in some experimental dogs, with temperatures returning to normal within 48 hours.<sup>6</sup> Persistence of fever has not been noticed clinically in dogs or cats.

Healthy dogs (n = 9) were monitored by pulse oximetry for 20 minutes following NB-BAL.<sup>37</sup> Supplementation with 100% oxygen through an endotracheal tube was maintained during the first 10 minutes. Oxygen saturation decreased below 90% in only one dog at a single time point (Sao<sub>2</sub> = 87%). No clinical signs of complications were noted, and thoracic radiographs were unremarkable 48 hours post-BAL.

Healthy cats (n = 4) were monitored by measuring arterial blood gases for 2 hours following NB-BAL.<sup>38</sup> While the cats were breathing room air, partial pressures of oxygen dropped from a mean of 81 mm Hg prior to BAL to 58 mm Hg 3 minutes post-BAL. Values steadily increased to a mean of 62 mm Hg at 10 minutes and 70 mm Hg at 20 minutes. The mean value had returned to baseline (83 mm Hg) at 1 hour postlavage. The decrease in oxygen tension was prevented with supplementation with 100% oxygen through an endotracheal tube, as evidenced by a mean oxygen pressure of 263 mm Hg 3 minutes post-BAL.

In the clinical situation, however, patients undergoing BAL have some degree of pulmonary compromise and may be susceptible to more severe decreases in lung function. Nevertheless, BAL is generally considered a safe procedure in humans with lung disease and is routinely done on an outpatient basis. Supplemental oxygen and monitoring by pulse oximetry and ECG are recommended.<sup>3</sup> Human patients with a history of asthma are premedicated with bronchodilators.<sup>3</sup> Complications of BAL in dogs and cats with lung disease without dyspnea are rare. In a retrospective study, 2 of 101 dogs (2%) undergoing BAL at a referral hospital died.<sup>36</sup> Both dogs were overtly dyspneic prior to BAL, and in one dog, additional invasive diagnostic procedures were also performed at the time of BAL. At necropsy both dogs had extensive multisystemic disease. During the same period, an additional 47 dogs with lymphoma (66% with lung involvement) were lavaged with no complications.<sup>33</sup>

Tracheal tear due to overinflation of the endotracheal tube cuff is a potential complication of NB-BAL in cats.<sup>39</sup> An endotracheal tube of sufficient size should be used, and care should be taken while inflating the cuff to create an airtight seal. Excessive inflation of the cuff must be avoided.

It is theoretically possible to rupture a cavitory lesion during BAL, although to the author's knowledge this has not been seen as a complication in dogs or cats. It is pru-

dent to decrease the volume of saline infused per bolus in patients with suspected cavitory lesions.

## Contraindications

Ideally, BAL is performed in patients that show no evidence of respiratory distress while breathing room air. Although the hypoxemia that occurs with BAL is transient and responsive to oxygen supplementation, patients must be able to tolerate general anesthesia and additional respiratory compromise. Bronchoalveolar lavage should not be performed in animals that have overt respiratory distress in spite of supplementation with oxygen. Relative risks and benefits of the procedure must be considered carefully in patients that fall between these extremes. Before any clinical patient undergoes BAL, the veterinarian should be prepared to provide supplemental oxygen for up to 2 hours following the procedure if necessary.

## Technique

Collection of high-quality BAL fluid requires that a sufficient volume of saline is instilled into an airway to reach the alveoli connected to that airway. In addition, a snug fit between the airway and the bronchoscope or lavage catheter must be achieved so that fluid from the deep lung is retrieved. A variety of techniques are available to achieve these goals. For consistency in results, standard protocols should be followed as much as possible. The techniques described below are protocols that are used by the author and for which cytological values from healthy dogs and cats have been published.

Monitoring of clinical patients during BAL should include continuous assessment of respiration, heart rate (via electrocardiography), and mucous membrane color. Whenever possible, pulse oximetry should be utilized to assess oxygen saturation, and arterial blood pressure should be monitored. Cats with historical or suspected airway disease should be treated prior to BAL with a bronchodilator to avoid bronchospasm associated with airway manipulation. Aminophylline (not sustained release; cats, 5 mg/kg; dogs, 11 mg/kg) can be given orally 1 or 2 hours prior to anesthesia, or terbutaline can be administered subcutaneously (cats, 0.01 mg/kg; can be repeated) 30 minutes prior to BAL.

### BRONCHOSCOPIC BAL

Bronchoalveolar lavage performed through a flexible bronchoscope allows sampling of specific lung lobes. The bronchoscope, including the channel, must be sterilized. Routine bronchoscopic examination of the airways is performed first to assist in the selection of grossly abnormal lobes for BAL, and because saline remaining in the airways following BAL will interfere with visualization. In every case, it is recommended that several lobes be lavaged to increase the diagnostic yield.<sup>31,36</sup> In compromised patients, preoxygenation with 100% oxygen is suggested for several minutes prior to BAL.

For each lobe that is to be lavaged, the bronchoscope is passed into successively smaller airways until a snug fit is achieved between the scope and the airway. It may be necessary to reposition the scope into an adjacent airway, still within the desired lobe, to find a good fit. The author routinely uses a 4.8-mm outer diameter pediatric bronchoscope, which generally lodges in a mainstem or lobar bronchus of cats, and 2 or 3 generations lower in most dogs.

Sterile, nonbacteriostatic, 0.9% sodium chloride (saline) solution is instilled through the biopsy channel of the bronchoscope into the airway by preloaded syringe. Immediately on completion of instillation, suction is applied to the same syringe to recover BAL fluid. If negative pressure is obtained rather than a return of fluid, less suction is applied to the syringe to minimize airway collapse. If negative pressure remains a problem, the scope is withdrawn a few millimeters. Airway collapse is most often a problem in dogs with chronic airway inflammation (bronchitis). If the scope is withdrawn too far, it will no longer be snug within the airway into which the fluid was instilled and subsequent suction attempts will produce mostly air. Vacuum suction and a specimen trap can be used instead of a syringe for fluid recovery, but it is more difficult to control the degree of suction and cells are more likely to become damaged in the process. After retrieval of as much fluid as possible from the first bolus of saline, the process is repeated for one or more additional boluses. Additional lobes are then lavaged using the same procedure. Following completion of BAL, 100% oxygen is provided through the endotracheal tube.

The volume of saline instilled has not been standardized in either people or animals. In humans, saline is instilled until a sufficient volume of fluid has been retrieved to perform indicated tests. Common total volumes are 100 to 300 ml/lobe.<sup>3</sup> The volume per bolus is limited by the volume of lung below the obstructed airway to avoid barotrauma. The number of boluses used is not critical as long as more than one bolus is used and consistent technique is followed. Fluid retrieved from the first bolus has the largest contribution of material from the large airways. It generally has a lower total cell count, increased neutrophils, and increased epithelial cells compared with fluid from subsequent boluses.<sup>7,37,40-42</sup>

In dogs, using a 4.8-mm outer diameter scope, the author routinely uses two boluses of 25 ml (50 ml total) in each lung lobe lavaged. In dogs less than 8 kg and cats or if using a smaller diameter scope that lodges more deeply within the lung (therefore leaving fewer alveoli to be filled), the author decreases the volume of fluid to 10 ml/bolus and instills four or five boluses per site. Regardless of the volume per bolus, preloaded syringes are used.

Indications of an excellent quality specimen include the presence of foam rising to the top of the fluid and a recovered volume of fluid exceeding 50% of the volume instilled (Figure 17-1). In cases with severe airway collapse, it may be impossible to achieve good fluid recovery. Additional boluses may increase the chances of ob-



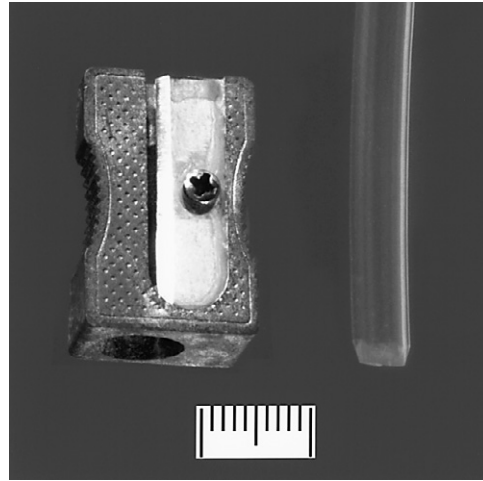
**Figure 17-1.** Foam floating on top of the specimen is one indication of successful sampling of material from the alveoli.

taining a representative specimen from the deep lung. In a retrospective study of BAL in clinical canine patients using two boluses of 25 ml each per lobe, the mean return volume was 24 ml (48%).<sup>36</sup>

### NONBRONCHOSCOPIC BAL IN DOGS

Nonbronchoscopic BAL in dogs can be performed using an inexpensive (approximately \$1) feeding tube.<sup>37</sup> The NB-BAL catheter is prepared as follows, maintaining sterile technique throughout. A 122-cm, 16 Fr Levin-type polyvinyl chloride stomach tube (Argyle stomach tube, Sherwood Medical Co, St. Louis, MO) is shortened by cutting off both ends. The distal end is cut off to eliminate the side openings. The proximal end is cut off to remove the flanged end and to decrease the total length of the tube. The final length should be approximately, but no shorter than, the distance from the open end of the dog's endotracheal tube to the last rib. Recovered fluid volume can be improved by slightly tapering the distal end of the tube using a simple, metal, single-blade, hand-held pencil sharpener that has been autoclaved and is used only for this purpose (Figure 17-2). A standard syringe adapter is attached to the proximal end of the NB-BAL catheter for attaching the syringes of saline.

The dog is anesthetized using a short-acting protocol that will allow intubation. Short-acting barbiturates, propofol, or the combination of medetomidine and butorphanol can be used following premedication with glycopyrrolate or atropine. The dog is intubated using a



**Figure 17-2.** Preparation of the catheter for performing non-bronchoscopic bronchoalveolar lavage in dogs. The metal, hand-held pencil sharpener has been autoclaved. The fenestrated end of the 16 French stomach tube has been cut off. The pencil sharpener is used to make a slight taper on the end of the tube to facilitate the tube fitting snugly within an airway lumen. Sterile technique is maintained.

sterile endotracheal tube. Intubation is carried out as cleanly as possible to minimize oral contamination of the specimen. The dog is positioned in dorsal recumbency. 100% oxygen is provided for several minutes through the endotracheal tube.

The NB-BAL catheter is passed through the endotracheal tube. In compromised patients, continued oxygen delivery can be achieved by passing the NB-BAL catheter through a bronchoscope swivel port. The NB-BAL catheter is passed into the airways until resistance is felt. The tube is withdrawn a few centimeters, then passed again until resistance is felt consistently at the deepest level to achieve a snug fit within an airway. Rotating the tube slightly on its axis may also facilitate lodging within an airway rather than abutting an airway division.

With the tube held in place, boluses of 25 ml of saline are instilled through the catheter immediately followed by 5 ml of air to minimize dilution of the specimen by the relatively large volume of the catheter relative to a bronchoscope channel. This process is greatly facilitated by prefilling 35-ml syringes with 25 ml of saline and 5 ml of air. The syringe is held upright during instillation so that the air follows the saline into the catheter (Figure 17-3). Immediately after instillation, suction is applied by the same syringe. If negative pressure is obtained, suction pressure is decreased. If negative pressure persists, the catheter is slowly withdrawn until fluid begins to appear in the syringe. If the catheter is withdrawn too far, it will no longer be snug within the airway into which the fluid was instilled and subsequent suction attempts will produce mostly air. The procedure is repeated for at least one more bolus of saline (50 to 75 ml total). Following BAL the dog is administered 100% oxygen through the endotracheal tube.



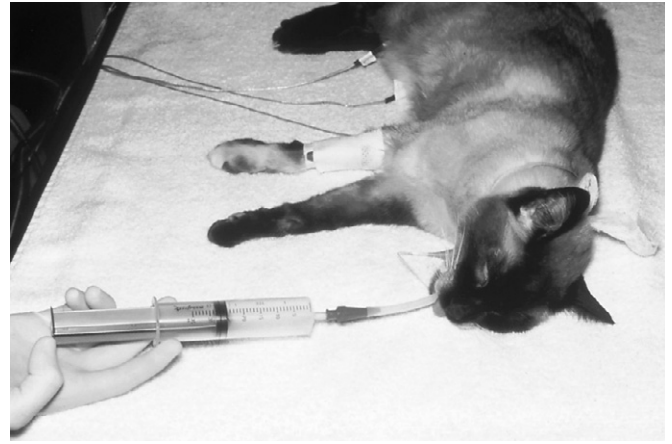
**Figure 17-3.** Performing nonbronchoscopic bronchoalveolar lavage in a dog. The dog is in dorsal recumbency. The lavage catheter has been passed until it is wedged within an airway. The syringe contains saline and air and is held upright so that the saline is infused first, followed by the air.

Based on mean recovery volumes from 9 healthy dogs, expected return from the first bolus is 44%, from the second bolus is 60%, and from a third bolus is 68%.<sup>37</sup> Decreased return can be anticipated in dogs with airway collapse.

Although this method of NB-BAL does not allow for directed specimen collection, the technique resulted in lavage of the right caudal lung lobe in 7 of 9 dogs and of the left caudal lung lobe in 2 dogs, based on the catheter position identified by thoracic radiographs prior to BAL. Therefore this technique is likely of most value for dogs with disease involving the caudal lobes. If disease is apparently localized to a specific caudal lobe, radiographic identification of catheter position and random attempts at replacement would likely allow for specimen collection from that lobe.

The specific feeding tube described was selected because it is readily available, of minimal expense, is sufficiently stiff to prevent folding or kinking within the endotracheal tube or airways and for the operator to feel resistance, and is sufficiently pliable to travel within the airways to lodge snugly in an airway lumen. In addition, the 5.3-mm diameter of the feeding tube is comparable to the outer diameter of bronchoscopes used in studies reporting BAL fluid cytology in dogs and should lodge in a similar generation of airways.<sup>8,36,43,44</sup> The NB-BAL catheter can be passed through an endotracheal tube as small as size 6, and the technique is therefore applicable to most dogs.

For dogs that are too small for this technique, the NB-BAL catheter can be passed directly through the larynx. Increased oral contamination is expected, but care can be taken to minimize contamination during passage. Where results of bacterial culture are critical, guarded bronchoscopic culture swabs can be passed blindly for specimen collection. Alternatively, a smaller diameter tube can be used to perform lavage.



**Figure 17-4.** Performing nonbronchoscopic bronchoalveolar lavage in a cat. The cuff of the endotracheal tube is carefully inflated to create a seal. The saline is infused into the tube using a standard syringe adapter. The procedure must be performed without delay once the syringe adapter is in place.

### NONBRONCHOSCOPIC BAL IN CATS

Nonbronchoscopic BAL in cats is easily performed through a sterile endotracheal tube. The cat is premedicated with glycopyrrolate or atropine. Short-acting anesthesia that will allow intubation is induced using standard protocols such as with ketamine and diazepam. Intubation is performed carefully to minimize oropharyngeal contamination. To ensure a clean intubation, lidocaine should be applied topically to the larynx and a laryngoscope used in every case. The cuff of the endotracheal tube is expanded to ensure an airtight seal, but overinflation of the cuff must be avoided to prevent tracheal tear.<sup>39</sup> In general, less than 3 ml of air should be needed for an appropriately sized tube.<sup>39</sup> The cat is placed in lateral recumbency. If the disease process is asymmetrical, than the most affected side should be placed against the table. Using this technique the majority of the lavage fluid is probably obtained from the cranial/middle lung lobes on the dependent side.

Following preoxygenation for a few minutes with 100% oxygen, the anesthetic adapter is removed from the endotracheal tube and replaced with a standard syringe adapter. At this point BAL must be performed quickly because the cat cannot ventilate adequately through the narrow syringe adapter lumen. Lavage is performed using three boluses of sterile saline, each of a volume of 5 ml/kg body weight. For a 4-kg cat, each bolus is 20 ml for a total lavage volume of 60 ml. The bolus is instilled through the endotracheal tube and immediately retrieved by suction (Figure 17-4). The procedure is rapidly repeated for each bolus. Cats generally take one or two breaths between each bolus.

Immediately after the procedure, the syringe adapter is removed and the cat's hindquarters are elevated for a few moments to allow additional fluid to drain from the

endotracheal tube. Then the anesthetic adapter is replaced on the tube and 100% oxygen is provided.

Based on mean retrieved volumes from healthy cats, approximate expected return volumes from this method of NB-BAL are 32% from the first bolus, 57% from the second, and 80% from the third.<sup>45</sup> A technique for NB-BAL in cats using a feeding tube has also been described.<sup>46,47</sup>

### POST-BAL PATIENT CARE

Immediately after BAL all patients are administered 100% oxygen by endotracheal tube for 5 to 10 minutes. Gentle positive pressure ventilation using the anesthesia reservoir bag may facilitate the opening of collapsed alveoli, as may positioning the patient in sternal recumbency. The patient is observed carefully for several minutes following discontinuation of oxygen supplementation. If pallor of mucous membranes is observed or measured oxygen saturation decreases, oxygen administration is reinstated. Attempts are made to discontinue the oxygen every 5 minutes. If the patient begins to recover from anesthesia before it is possible to discontinue oxygen administration through the endotracheal tube, a decision must be made whether or not to use gas anesthesia to allow continued control of the airway. In all but severely compromised patients, the patient can be allowed to recover and the endotracheal tube removed. In most of these cases, oxygen supplementation via face mask or oxygen cage is sufficient to maintain adequate oxygenation. It is rare for previously stable patients to require more than 5 to 10 minutes of oxygen supplementation following BAL.

If a patient fails to respond to oxygen supplementation, the potential for bronchospasm or pneumothorax should be considered. Bronchospasm is most likely to occur in cats with reactive airway disease. Wheezes may be auscultated and increased expiratory efforts observed. Bronchodilators should be administered. Pneumothorax could occur secondary to rupture of a cavitary lesion or, rarely, from a tracheal tear related to intubation and cuff overinflation. The latter generally causes subcutaneous emphysema. If decreased lung sounds are auscultated, therapeutic thoracocentesis should be performed. Pneumothorax as a result of BAL has not been observed by the author.

Retained fluid is isotonic and is absorbed from the alveoli; however, it is normal to auscult crackles for up to 24 hours after BAL.<sup>6</sup> Radiographic evidence of fluid and atelectasis should resolve within 2 days.<sup>6,37</sup>

### Specimen Processing

Fluid should be kept in plastic or silicone-treated syringes or tubes pending analysis because phagocytes adhere readily to glass. Fluid for culture should be placed promptly in appropriate transport media. Fluid for cytological analysis should be processed within 1 hour for optimal results.<sup>48</sup> Specimens that cannot be processed soon after collection should be refrigerated and processed within 12 hours.<sup>3</sup>

### WHEN TO COMBINE BAL FLUID FOR ANALYSIS

It is advisable to process BAL fluid from different lobes separately for cytological evaluation. In a retrospective study of BAL in dogs, additional cytological information was obtained by evaluating multiple lobes in about one third of cases even though diffuse disease was indicated radiographically.<sup>36</sup> A study of BAL cytology from dogs with fungal pneumonia showed organisms were not visible in every lobe lavaged in 3 out of 6 dogs in which organisms were identified cytologically.<sup>31</sup> In addition, the author has found that fungal and protozoan infections may only manifest one visible organism on an entire slide.<sup>31,34</sup> Preparing slides from multiple lobes results in examination of increased numbers of slides for organisms in low concentrations. It is acceptable to combine fluid from all lobes sampled for culturing unless the disease appears to be localized to a specific lobe. In this instance, unnecessary dilution would occur.

It is generally appropriate to combine BAL fluid from separate boluses from the same lung lobe. In human medicine, some pulmonologists advocate discarding return from the first bolus because of its relatively greater representation from the larger airways.<sup>2,3</sup> Others believe that this practice is unnecessary.<sup>49</sup> In dogs and cats, it is unlikely that the minor contribution of the large airways to a BAL specimen would be clinically relevant. However, if the underlying disease process primarily affects the large airways and not the deep lung, then the dilution of material from the large airways by material from the deep lung might affect results. For instance, in one dog with chronic bronchopneumonia, only *Bordetella* was recovered from a tracheal wash, whereas only *Pseudomonas* was recovered from a BAL specimen. If large airway disease is suspected, either the fluid returned from the first bolus of BAL should be processed separately or a specifically directed airway specimen (e.g., bronchial wash, tracheal wash, bronchial brushing, or bronchial biopsy) should be submitted in addition to the BAL specimen.

### CYTOLOGICAL EXAMINATION

Total nucleated cell counts are performed on undiluted BAL fluid using a hemocytometer. The cell sizes prevent accurate results using automated counters. The concentration of cells in BAL fluid is often too dilute for evaluation of direct smears. Instead, concentrated preparations must be made using techniques such as cytocentrifugation, which provides high quality slides. Volumes of 100 to 200  $\mu\text{l}$ /slide are generally required. Wright-Giemsa or quick Romanowsky stains are used routinely. Special stains to further characterize abnormal cell populations or to facilitate the identification of organisms may be useful in some cases. Fluid should not be strained through gauze prior to processing to remove mucus strands because certain cells or organisms may be selectively retained.

Cytological characterization includes the performance of differential cell counts. A minimum of 200 cells should be counted, and qualitative changes are noted. Macrophages are examined for evidence of activation and for phagocy-

tized organisms, debris, red blood cells, or hemosiderin. Neutrophils are examined for degenerative changes and intracellular organisms. All cells are examined for criteria of malignancy. As with any cytological specimen, care must be taken in interpreting criteria of malignancy in the face of inflammation. The entire slide should be carefully scrutinized for the presence of infectious agents. Only one organism may be present on the slides of a patient with fungal, protozoal, or parasitic disease.

### BACTERIAL CULTURE

Ideally, quantitative or semiquantitative culturing methods should be employed for BAL fluid. The large airways are not completely sterile in health, and some oral contamination can occur during the procedure. The determination of the significance of cultured bacteria is further complicated by the presence of increased numbers of organisms within the airways of patients with reduced airway clearance (particularly chronic bronchitis) relative to healthy patients. The significance of these organisms is not known, but overt inflammation and clinical signs are not always present. On the other hand, BAL dilutes the concentration of any pathogens that are present.

To minimize costs it is common in veterinary medicine to employ routine culturing techniques. If quantitative cultures are not performed, lavage fluid should be inoculated directly onto culture plates, as well as inoculated into enrichment media to identify slow-growing organisms and organisms present in low numbers.

### MYCOPLASMA CULTURE

The role of *Mycoplasma* in respiratory disease of the dog and cat is still not well characterized. A role for the organisms as a cause for lung disease in cats has been proposed, and the organisms have been cultured from BAL fluid of cats with pneumonia.<sup>50,51</sup> As mucosal inhabitants, bronchial brushings or washings may provide a superior specimen to BAL. Specific handling and culturing techniques are necessary.

### FUNGAL CULTURE

Culture of BAL fluid for fungal organisms improves the sensitivity of the technique for diagnosing fungal pneu-

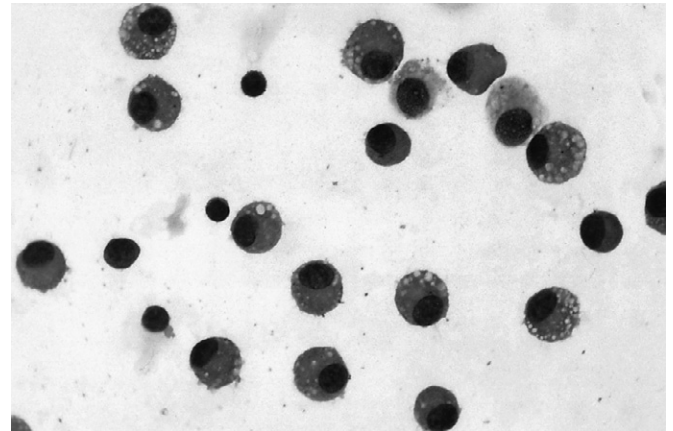
monia.<sup>52</sup> Cultures should only be performed in laboratories equipped to handle these organisms.

## Interpretation of Results

### CYTOLOGY

A great deal of variability is present among healthy dogs and cats with regard to BAL fluid cell counts (Table 17-1). Slight deviations compared with normal values should not be overinterpreted. Increased variability is added by inconsistency in collection techniques and specimen processing. Regardless, the predominant cell type in health is the alveolar macrophage (Figure 17-5). Lymphocytes can be difficult to distinguish cytologically from small macrophages, and variability in expected numbers has been reported.<sup>53</sup> Immunofluorescent, immunocytochemical, and flow cytometric techniques can be used for accurate differentiation of mononuclear cells. Neutrophils are present in slightly greater numbers in fluid returned from the first bolus in dogs and people.<sup>7,37, 40-42</sup>

Eosinophil counts can be high in clinically healthy cats and dogs.<sup>44,54</sup> Eosinophil counts from the same cats lavaged eight times at intervals of 1 or more weeks varied by 1% to 52% compared with values from their first lavage.<sup>55</sup> Therefore, the finding of relative eosinophilia



**Figure 17-5.** Bronchoalveolar lavage fluid from a healthy dog. The predominant cell type is the alveolar macrophage. (Wright-Giemsa, 250 $\times$ )

**TABLE 17-1. Reported Cell Counts of BAL Fluid From Healthy Dogs and Cats Using the Described Collection Techniques**

	Canine B-BAL <sup>58</sup> (Mean $\pm$ SD)	Canine NB-BAL <sup>37</sup> (Mean $\pm$ SD)	Feline B-BAL <sup>63</sup> (Mean $\pm$ SE)	Feline NB-BAL <sup>45</sup> (Mean $\pm$ SD)
TNCC (/ $\mu$ l)	200 $\pm$ 86	352 $\pm$ 115	241 $\pm$ 101	337 $\pm$ 194
Macrophages (%)	70 $\pm$ 11	81 $\pm$ 11	71 $\pm$ 10	78 $\pm$ 15
Neutrophils (%)	5 $\pm$ 5	15 $\pm$ 12	7 $\pm$ 4	5 $\pm$ 5
Eosinophils (%)	6 $\pm$ 5	2 $\pm$ 3	16 $\pm$ 7	16 $\pm$ 14
Lymphocytes (%)	7 $\pm$ 5	2 $\pm$ 5	5 $\pm$ 3	0 $\pm$ 1

TNCC, Total nucleated cell count; SD, standard deviation; SE, standard error.



should be interpreted with careful consideration also given to the total nucleated cell count, patient history, and other clinical data. Mast cells are usually less than 1% to 2% of white blood cells in normal dogs.<sup>56,57</sup>

Epithelial cells are usually less than 5% of nucleated cells, and greater numbers suggest a large contribution to the fluid from the large airways.<sup>3</sup> An exception is fluid collected using the NB-BAL technique described for dogs. As many as 2 to 5 clumps of epithelial cells/hpf were noted in fluid from the first boluses, presumably from disruption of cells during efforts to blindly lodge the lavage catheter within the airways.<sup>37</sup>

Most emphasis is placed on the relative cell counts when identifying an inflammatory response of the lungs. The relative counts are independent of the variability in dilution inherent in BAL specimens. It is not possible to accurately assign specific numbers to distinguish normal from abnormal relative cell counts because of the variability seen in health. Criteria used to identify abnormal inflammatory responses in dogs reported in the previously mentioned retrospective study were based on the 90th percentile values of relative cell counts from BAL fluid collected using the bronchoscopic method from 30 histologically normal lung lobes from five healthy dogs.<sup>36,58</sup> Based on these criteria, relative neutrophil counts greater than 12% are considered to indicate neutrophilic inflammation, eosinophil counts greater than 14% are considered to indicate eosinophilic inflammation, and lymphocyte counts greater than 16% are used to indicate lymphocytic inflammation.

In clinical practice, total nucleated cell counts are also used to temper the interpretation of increases in relative counts. Very low total nucleated cell counts may indicate that the fluid collected is more likely a bronchial wash than BAL fluid. Marginally increased relative cell counts in combination with a low total nucleated cell count are not as likely to be clinically significant as those associated with an increased total nucleated cell count.

Because macrophages normally make up the majority of cells in BAL fluid, an increase in these cells is only identifiable by increased cell numbers. However, clinically relevant pulmonary inflammation can be expected to result in a concurrent increase in relative neutrophil, eosinophil, or (occasionally) lymphocyte counts. Macrophagic inflammation is better assessed by cytological criteria of differentiation (e.g., increased size, less basophilic cytoplasm, vacuolization, and phagocytized debris).<sup>59</sup> Macrophage activation can be seen in clinically normal dogs and cats, particularly if they are exposed to smoke, smog, or other particulates. As with eosinophilia, this finding must be interpreted with consideration of other available information.

Neutrophils are scrutinized for evidence of degenerative change (e.g., pyknotic, karyorrhectic, or karyolytic nuclei). The presence of these changes is supportive of a septic process; however, it is not uncommon for the neutrophils to appear normal in the face of infection.<sup>59</sup>

Macrophages and neutrophils are scrutinized for the presence of intracellular organisms. Intracellular bacteria indicate active infection. Intra- or extracellular pathogenic fungal organisms, parasite larvae or ova, or protozoal organisms are diagnostic for active infection.

Slides are scanned for abnormal cell populations. As with other cytological specimens, criteria of malignancy may represent hyperplasia and metaplasia rather than neoplasia if an inflammatory response is present.

## BACTERIAL CULTURES

Large airways can be inhabited by bacteria in health, and increased numbers can be expected in patients with reduced airway clearance (a complication of diseases such as chronic bronchitis, bronchiectasis, and ciliary dyskinesia). Therefore, growth of bacteria from BAL fluid does not necessarily indicate infection. In people, growth of greater than  $10^5$  colonies/ml of fluid is associated with pneumonia. Growth of greater than  $10^3$  colonies/ml may represent infection, particularly in patients that have recently received antibiotics.<sup>60</sup> A study involving 47 dogs determined that bacterial growth of  $1.7 \times 10^3$  colonies/ml was associated with airway infection.<sup>61</sup> Results of BAL fluid cultures correlate well with results from specimens collected with guarded catheter swabs, although slightly lower thresholds are used to diagnose pneumonia when swabs are used.<sup>60</sup>

Quantitative cultures are rarely performed in veterinary medicine. Infection is confirmed if intracellular bacteria are identified cytologically. The growth of organisms on agar plates inoculated directly with BAL fluid has also been considered to represent infection.<sup>62</sup> Infection is unlikely in the absence of neutrophilic inflammation. The cases that pose a diagnostic problem are those with neutrophilic inflammation, no intracellular bacteria, and growth of organisms only following incubation of BAL fluid in enrichment media. These cases may have true infection but have relatively low numbers of organisms because of the dilution of the specimen or because of recent treatment with antibiotics. Alternatively, these cases may not have true infection.

Unfortunately, in veterinary medicine BAL is often not recommended until after antibiotics fail to resolve the clinical signs. Antibiotics within the lung may persist in sufficient concentrations to interfere with culturing efforts for as long as 1 week (and probably longer with some of the newer, longer-lasting antibiotics) even when these antibiotics have been clinically ineffective. The clinical condition of the patient may not allow a delay before BAL is performed. Therefore, BAL fluid should also be placed in enrichment broth to promote the growth of bacteria present in low numbers. The significance of positive cultures must then be interpreted with caution. This diagnostic dilemma can often be avoided by performing BAL prior to initiation of antibiotics and by collecting high-quality specimens using careful techniques to minimize contamination.

## Conclusion

Bronchoalveolar lavage is a clinically useful technique for obtaining specimens from the lung. Its diagnostic value for dogs and cats with pulmonary disease will continue to grow as we obtain increased experience with the tech-

nique and as sensitive and specific laboratory methods applicable to the collected fluid continue to be developed.

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## CHAPTER 18

# Tracheal Washes

Rebecca S. Syring

### *Background and Definition*

The tracheal wash is a minimally invasive diagnostic technique used to sample the respiratory tract of dogs and cats. Tracheal washes are used primarily to obtain samples from the large airways (trachea and primary bronchi) and are considered less helpful in the diagnosis of interstitial or alveolar lung disease. Specimens obtained from tracheal washes can be evaluated cytologically to identify and characterize the inflammatory response and to identify any infectious agents or neoplastic cells. Bacterial or fungal cultures can be performed on these specimens to confirm an infectious etiology.

Tracheal washes can be performed by either a transtracheal or endotracheal route. It has been suggested that the transtracheal wash (TTW) may be superior to an endotracheal wash (ETW) for sampling smaller airways and alveoli. Because sedation is usually not required for a TTW, the patient's cough reflex remains intact during the procedure, therefore potentially providing a sample from the smaller airways and alveoli. To the author's knowledge, no studies have been published comparing the diagnostic yield of TTW to ETW.

A few clinical studies have compared the diagnostic yield of endotracheal washes with bronchoalveolar lavage (BAL), in which ETW immediately preceded BAL.<sup>1-3</sup> In

dogs with multicentric lymphoma, pulmonary involvement was detected in 4 of 41 dogs via ETW.<sup>3</sup> Although lymphoma was also detected via BAL in all 4 of these dogs, pulmonary involvement was documented in 23 additional dogs using BAL.<sup>3</sup> Similarly, in 9 dogs that had systemic fungal infections with suspected pulmonary involvement, ETW was successful in identifying *Blastomyces* in 3 dogs. However, in this same population, BAL isolated *Blastomyces* in 5 dogs and *Histoplasma* in 1 dog.<sup>2</sup> A case report of a cat with pulmonary *Cryptococcus* stated that infectious agents were detected in both the ETW and BAL, however the ETW contained fewer organisms.<sup>1</sup> These studies suggest that although tracheal washes may provide useful diagnostic information, they are less sensitive than BAL.

Only one clinical study investigates TTW compared with BAL; however, this study includes both TTW and ETW into a general category of tracheal washes and does not report results individually.<sup>4</sup> In this study, both a tracheal wash (TTW or ETW) and a BAL were performed in 66 dogs. The cytological interpretation of the samples retrieved differed between procedures in 68% of dogs. In this study, BAL more often detected hemorrhage, infectious agents, and neoplasia compared with tracheal washes. In addition, the cytological pattern of inflammation differed in 41% of animals between the two pro-