Effect of repetitive bronchoalveolar lavage on cytologic findings in healthy dogs

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Objective—To determine reference values for cytologic examination results of bronchoalveolar lavage fluid (BALF) and to investigate effects of repeated lavages on pulmonary health and on results of cytologic examination of BALF in dogs.

Animals—16 healthy adult Beagles.

Procedure—All dogs underwent pulmonary lavage to obtain BALF. Eleven dogs were repeatedly lavaged 6 times at 5- to 7-week intervals. Analyses for total and differential cell counts and for viability of cells before and after cell processing were performed. Arterial blood gas analysis before and after bronchoalveolar lavage was used to study the safety of the lavage procedure. Histologic and radiologic examinations were used to study effects of repeated lavages on pulmonary health.

Results—Mean (± SD) cell count was 104 ± 69 cells/µl, comprising 75 ± 7% alveolar macrophages, 13 ± 6% lymphocytes, 5 ± 4% neutrophils, 4 ± 5% eosinophils, 2 ± 2% mast cells, 0.6 ± 0.7% epithelial cells, and 0.3 ± 0.4% plasma cells. Centrifugation of samples and washing of cells caused significant cell loss (59 ± 13%). Repeated lavages did not cause significant variations in cell counts of BALF or results of arterial blood gas analysis, thoracic radiography, or histologic examination of pulmonary specimens. Only a moderate, although significant, decrease in arterial oxygen content was observed after bronchoalveolar lavage.

Conclusions and Clinical Relevance—Analysis indicated that several lavages performed at 5- to 7-week intervals can safely and reliably be used to study the kinetics of pathologic processes in pulmonary tissues or for evaluation of therapeutic efficacy. (Am J Vet Res 2001;62:13–16)

Bronchoscopy and bronchoalveolar lavage (BAL) have been used for diagnostic and research purposes in small animals, humans, and horses.1-3 Despite wide use, a consensus has not been reached for the technical aspects, such as differing lavage routines, fluid volumes, and laboratory techniques that are in use. Therefore, several reference values exist for dogs,3,4,5 and results from investigators that use differing methods are difficult to compare.3,5

Samples obtained during sequential BAL from the same animal could be used to evaluate responsiveness to treatment or to investigate the pathogenesis of respiratory tract disease, but it is not known which changes in cell population, radiographic results, or histologic findings are representative of the disease under investigation and which changes are attributable to repeated BAL. Several investigators6,10,11 have studied the effects of repeated BAL in dogs, but the main aim of those studies was to develop the BAL procedure for humans, not to study the safety and reliability of BAL methods used to diagnose and study lung diseases in dogs.

Bronchoalveolar lavage usually is a safe procedure for the patient, causing only mild histologic changes in pulmonary tissues.5,11,12 In humans, acute transient pyrexia and bronchospasm may develop infrequently.11,12 A decrease in arterial oxygen pressure as a result of BAL has been reported in cats, dogs, and humans.5,12,13,14

One objective of the study reported here was to create cytologic reference ranges for healthy dogs, using the laboratory methods reported in another study,16 and to investigate the effects of repeated lavages. Additionally, the influence of centrifugation and washing of cells on cell counts and cell viability of samples were assessed. Safety of the lavage procedure for dogs and the effect of repeated lavages on the lungs were evaluated, using results from differential and total cell counts of BAL fluid (BALF), blood gas analysis before and after each lavage, thoracic radiography before and after the entire series of lavages, and histologic examination of the lavaged and nonlavaged parts of the lungs.

Materials and Methods

Dogs—Sixteen clinically healthy Beagles (11 females and 5 males), 3 to 10 years old that weighed between 8 and 18 kg, were used for the study. Dogs did not have clinical signs of disease. Dogs were routinely dewormed twice yearly. Clinical examination, hematologic examination, and serum biochemical analysis were performed before each lavage procedure.

Bronchoalveolar lavage procedure—All dogs underwent bronchoscopy and BAL. In 11 dogs, BAL was repeated at 5- to 7-week intervals.

For BAL, dogs were sedated with medetomidine (40 µg/kg of body weight, IM), and anesthesia was induced with propofol (1 mg/kg, IV). After laryngeal inspection, a fiberoptic bronchoscope was passed into the trachea. Tracheal and bronchial lumen, and mucous membrane, were inspected for abnormalities. The tip of the scope was wedged into 1 of the caudal lobes of the lungs, and sterile warmed (37°C) saline (0.9% NaCl) solution (2 ml/kg, divided in 2 aliquots) was infused through the biopsy channel. Three milliliters of the infused fluid remained in the biopsy channel of the broncho-
scope. Gentle suction was applied, and retrieved fluid was placed in a glass container kept on ice. The procedure was repeated for the other caudal lobe; therefore, the total fluid volume was 4 ml/kg. Samples from both caudal lobes were pooled. The dogs did not receive supplemental oxygen during the BAL procedure. Sedation was reversed with atipamezole (200 µg/kg, IM). Dogs recovered within 5 to 10 minutes after injection of atipamezole and were able to walk out of the examination room.

Cytologic examination of BALF—Pooled BALF samples were filtered through a 1-layer cotton gauze. The recovered volume was measured and expressed as percentage of total infused fluid. The BALF cell count and viability of cells were determined by use of a hemocytometer, using trypan blue stain (1:1). The remaining portion of each sample was centrifuged (100 X g for 10 minutes), and the cell pellet was washed with 10 ml of PBS solution. After a second centrifugation, the cell pellet was resuspended in 1 ml of PBS solution. The cell count and viability determination were repeated, and the result was expressed in corresponding volumes, similar to the manner used for the original BALF. An aliquot containing 40,000 cells was cyt centrifuged (250 X g for 10 minutes) on slides and stained with May–Grünewald–Giemsa stain. Three hundred cells were counted, and differential counts for macrophages, lymphocytes, neutrophils, eosinophils, plasma cells, mast cells, and epithelial cells were determined.

Effects of repetitive lavages—Two arterial blood samples were collected from a femoral artery of each dog. The first sample collected for arterial blood gas analysis was obtained before sedation, and the second sample was obtained within 5 minutes after BAL but before reversal of sedation. The arterial–alveolar gradient (A-aDO₂) was calculated.

Lateral and ventrodorsal thoracic radiographs were obtained prior to the first BAL for all dogs. For the 11 dogs in the repetitive lavage group, radiography was repeated 2 weeks after the last BAL. Two examiners (MMR, AKJ), who were unaware of the status of the dogs, independently evaluated the radiographs. Five dogs, which were between 8 and 10 years old, in the repetitive lavage group were euthanatized 3 weeks after the last lavage. Tissue samples (3 samples from each lung lobe) were collected and fixed in 4% neutral-buffered formalin immediately after the dogs were euthanatized. Samples were obtained from cranial (nonlavaged) and caudal (lavaged) lobes for histologic evaluation of possible pathologic lung changes. Specimens were stained routinely with H&E and Masson-Trichrome stains, which were used to evaluate collagen formation. Health of the dogs was verified during complete postmortem examinations.

Statistical analysis—Reference ranges were calculated for values obtained for the 16 dogs. Values were expressed as mean ± SD and 95% confidence intervals (CI).

Results

Health status—Dogs remained healthy during the entire study; as determined on the basis of results of clinical examination, hematologic examination, and serum biochemical analysis that were repeated before every lavage. Clinical signs of pulmonary or other diseases were not detected. Bronchoscopy did not reveal abnormal findings of the airways. On the basis of thoracic radiography, the lungs were considered to be normal before the first BAL session, further strengthening our assessment of the health status of the dogs.

Cytologic examination of BALF—Mean (± SD) recovery rate of instilled fluid was 63 ± 8%. The recovered BALF contained 104 ± 69 cells/µl, and the resuspended cell pellet contained 62 ± 46 cells/µl. A significant (P = 0.001) amount of cells was lost during the 2 centrifugations and 1 washing (range, 2 to 88%; mean ± SD, 59 ± 13%). Cell loss was not dependent on the number of the BAL session or the dog involved. Correlation analysis indicated a significant strong positive correlation between the number of cells in the original BALF sample and the resuspended cell pellet (r = 0.87, P < 0.001). Alveolar macrophages were the dominant cells detected. In order of decreasing frequency, the remaining cells consisted of lymphocytes, neutrophils, eosinophils, mast cells, epithelial cells, and plasma cells. Mean ± SD and 95% CI for total and differential cell counts were determined (Table 1). We did not detect a correlation between body weight of a dog and cell counts in the original BALF sample.

Repeated lavages did not significantly affect cell counts or A-aDO₂, PaO₂, or PaCO₂ before BAL. However, significant variations were detected among BAL sessions for percentages of macrophages, lymphocytes, and mast cells; variations in percentages of neutrophils, eosinophils, plasma cells, and epithelial cells did not differ significantly. A significant difference in values between 2 successive sessions was found only among percentages of macrophages and eosinophils.

Table 1—Results for analysis of fluid obtained during bronchoalveolar lavage (BAL) of 16 healthy Beagles.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>95% Confidence interval</th>
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<tbody>
<tr>
<td>Body weight (kg)</td>
<td>13.4 ± 2.5</td>
<td>12.1—14.8</td>
</tr>
<tr>
<td>Recovered volume (%)</td>
<td>60.1 ± 1.5</td>
<td>59.1—61.1</td>
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<tr>
<td>Viability (%)</td>
<td>84.8 ± 17.9</td>
<td>76.1—93.4</td>
</tr>
<tr>
<td>Original sample</td>
<td>71.9 ± 11.3</td>
<td>65.3—76.7</td>
</tr>
<tr>
<td>Resuspended cell pellet</td>
<td>103.9 ± 68.5</td>
<td>67.4—140.5</td>
</tr>
<tr>
<td>Cell count (No. of cells/µl)</td>
<td>61.8 ± 46.3</td>
<td>37.2—86.5</td>
</tr>
<tr>
<td>Macrophages %</td>
<td>75.3 ± 6.9</td>
<td>71.8—78.7</td>
</tr>
<tr>
<td>cells/µl</td>
<td>76.4 ± 48.2</td>
<td>50.7—102.1</td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td>15.2 ± 5.8</td>
<td>10.3—16.1</td>
</tr>
<tr>
<td>cells/µl</td>
<td>15.0 ± 13.7</td>
<td>7.7—22.3</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>4.9 ± 3.8</td>
<td>2.9—6.9</td>
</tr>
<tr>
<td>cells/µl</td>
<td>6.4 ± 7.1</td>
<td>2.6—10.2</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>3.6 ± 4.7</td>
<td>1.2—6.0</td>
</tr>
<tr>
<td>cells/µl</td>
<td>3.5 ± 5.0</td>
<td>0.8—6.2</td>
</tr>
<tr>
<td>Mast cells %</td>
<td>2.2 ± 1.7</td>
<td>1.3—3.0</td>
</tr>
<tr>
<td>cells/µl</td>
<td>2.0 ± 1.8</td>
<td>1.0—3.0</td>
</tr>
<tr>
<td>Epithelial cells %</td>
<td>0.6 ± 0.7</td>
<td>0.2—0.9</td>
</tr>
<tr>
<td>cells/µl</td>
<td>0.6 ± 1.0</td>
<td>0.1—1.1</td>
</tr>
<tr>
<td>Plasma cells %</td>
<td>0.3 ± 0.4</td>
<td>0.1—0.5</td>
</tr>
<tr>
<td>cells/µl</td>
<td>0.2 ± 0.3</td>
<td>0.1—0.4</td>
</tr>
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*Values differ significantly (P < 0.01). †Values differ significantly (P < 0.001).
with mast cells (between BAL sessions 5 and 6), whereas all other differences were not associated with a specific dog or number of the BAL session.

Cell viability was 85 ± 17% in the original BALF samples. Cell viability was significantly (P = 0.01) decreased by 2 centrifugations and 1 washing to a value of 71 ± 11% (Table 1).

Arterial blood gas analysis—Mean ± SD of A-aDO2, PaO2, and PaCO2 were determined (Table 2). Mean PaO2 after BAL was significantly (P < 0.001) less than the PaO2 before sedation. Mean A-aDO2 (P < 0.001) and PaCO2 (P < 0.05) were significantly higher after BAL than the values before BAL. There was not a correlation between percentage of fluid recovered and A-aDO2 after BAL.

Thoracic radiography—Evaluation of thoracic radiographs did not reveal abnormal findings before the first lavage or after the series of repeated lavages.

Histologic evaluation—Only changes typical for lungs of aging dogs were detected.18 Enlarged distal air spaces and atrophic interalveolar septa were detected in all samples. Anthracotic pigment and some foci of interstitial fibrosis were detected, especially in cranial lung lobes. In 3 dogs, we detected minimal amounts of interstitial fibrosis, especially in cranial lung lobes. In 3 dogs, we detected minimal amounts of interstitial fibrosis, especially in cranial lung lobes. Neither arterial blood oxygen concentrations prior to centrifugation, speed, duration and number of centrifugations, washing of cells, method of cytocentrifugation, and methods used for cell counting all affect cellular results.2 These technical variations have led to several reference values for BALF of dogs, and, therefore, results from various studies are difficult to compare. Centrifugation of a sample and washing of cells reportedly diminish the cell numbers in BALF of humans by 18 to 34%.21,22 Lapointe et al1 reported for horses that 1 centrifugation does not cause significant cell loss, but notable variation was detected for individual horses. We are unaware of reports of the effects of centrifugation on cell numbers or viability of cells in BALF of dogs. In the study reported here, in which cells underwent 2 centrifugations and 1 washing, significant cell loss was detected, and there was large variation among dogs. Despite cell loss, total cell counts of the original BALF and the resuspended cell pellet were highly correlated. Therefore, both reflect cell numbers of the lavaged lobe, although separate reference values have to be used. Because of cell loss, a more reliable estimate of the cellular content of the lavaged lung surface can be obtained by use of the cell count from the nonprocessed original BALF.

Viability of cells might reflect health of the respiratory tract. Cell viability is especially important when the cells are to be used subsequently for culture.3 We noticed that processing of the cells significantly decreased their viability in healthy dogs, and it is possible that cell viability might change even more in pulmonary tract diseases, especially when diseased cells are processed.

Lavages can induce transient neutrophilia in BALF obtained from dogs, horses, sheep, and monkeys19-21,22, this neutrophilia lasts at least 48 hours.23,24 In those studies, repetition of lavages at 6- to 7-week intervals did not cause significant changes in cell counts. Incidental changes in relative counts of macrophages, lymphocytes, and mast cells during the study reported here did not reveal a decrease or increase in percentages that could be connected to the number of lavages. Neither arterial blood oxygen concentrations prior to BAL, radiologic findings of the lungs, nor results of histologic examination of pulmonary tissues were affected by repeated lavages. Thus, several repeated lavages of the same lung area at 5- to 7-week intervals did not cause permanent changes to the lavaged lung or to the basis of body weight.

Cell counts in our study were less than those in 2 studies in which investigators used higher volumes of lavage fluid and differing lavage procedures.23,24 Our results for differential cell count are comparable to those in studies that used healthy dogs.16,25 For differential cell counts, it is typical that the absolute values are rarely reported, compared with the relative values, which seem to be more constant. However, the use of relative values limits the interpretation of the actual numbers of certain cell types on the respiratory epithelial surface, especially when the total number of cells changes because of a disease process. Therefore, absolute and relative values should be used concurrently.

Additionally, differing laboratory techniques cause variation. Filtration of the sample to remove mucus prior to centrifugation, speed, duration and number of centrifugations, washing of cells, method of cytocentrifugation, and methods used for cell counting all affect cellular results.2 These technical variations have led to several reference values for BALF of dogs, and, therefore, results from various studies are difficult to compare. Centrifugation of a sample and washing of cells reportedly diminish the cell numbers in BALF of humans by 18 to 34%.21,22 Lapointe et al1 reported for horses that 1 centrifugation does not cause significant cell loss, but notable variation was detected for individual horses. We are unaware of reports of the effects of centrifugation on cell numbers or viability of cells in BALF of dogs. In the study reported here, in which cells underwent 2 centrifugations and 1 washing, significant cell loss was detected, and there was large variation among dogs. Despite cell loss, total cell counts of the original BALF and the resuspended cell pellet were highly correlated. Therefore, both reflect cell numbers of the lavaged lobe, although separate reference values have to be used. Because of cell loss, a more reliable estimate of the cellular content of the lavaged lung surface can be obtained by use of the cell count from the nonprocessed original BALF.

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cellular contents of BALF. Cellular results of our study are in agreement with results of Carre et al., although they lavaged their dogs only 3 times. On the basis of these findings, repeated BAL procedures can be safely performed in dogs. Thus, repeated lavage of the same lobe can be performed at 5- to 7-week intervals to monitor pulmonary tract disease without affecting cellular results.

The PaO2 decreases and A-aDO2 increases significantly as a result of the BAL procedure, including effects of BAL, sedation, and recumbency in healthy dogs. However, these changes can be more pronounced in dogs with pulmonary tract disease. A noticeable decrease in PaO2 and increase in A-aDO2 after BAL has also been reported in cats affected by various respiratory or gastrointestinal tract diseases. Interestingly, the changes of PaO2 in the study reported here were minimal, although we did not use supplemental oxygen, compared with reported changes in healthy dogs in which investigators used oxygen during the entire procedure. Thus, the lavage procedure we used, combined with sedation achieved by use of medetomidine and anesthesia induced by parenteral administration of propofol, proved to cause fewer changes in oxygenation of arterial blood and, therefore, should be considered safer than previously reported methods. Additionally, when sedation can be reversed with atipamezole, it alleviates the need for clinicians to monitor long recovery periods. Thus, in addition to being considered safer than previously reported methods, the BAL procedure reported here is more convenient for animals, clients, and veterinarians.

References

6. Cohen AB, Batra GK. Bronchoscopy and lung lavage