

Comparison of results for weight-adjusted and fixed-amount bronchoalveolar lavage techniques in healthy Beagles

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Objective—To compare recovery of epithelial lining fluid (ELF) in bronchoalveolar lavage fluid (BALF) by use of weight-adjusted or fixed-amount volumes of lavage fluid in dogs.

Animals—13 healthy Beagles.

Procedures—Dogs were allocated to 2 groups. In 1 group, the right caudal lung lobe was lavaged on the basis of each dog's weight (2 mL/kg, divided into 2 aliquots) and the left caudal lung lobe was lavaged with a fixed amount of fluid (50 mL/dog, divided into 2 aliquots). In the second group, the right and left caudal lung lobes were lavaged by use of the fixed-amount and weight-adjusted techniques, respectively. The BALF was collected by use of bronchoscopy. A recovery percentage $\geq 40\%$ was required. The proportion of ELF was calculated by use of the following equation: (concentration of urea in BALF/concentration of urea in serum) $\times 100$.

Results—Mean \pm SD proportion of ELF in BALF was $2.28 \pm 0.39\%$ for the weight-adjusted technique and $2.89 \pm 0.89\%$ for the fixed-amount technique. The SDs between these 2 techniques differed significantly (calculated by comparing 2 covariance structures [unstructured and compound symmetry] in a repeated-measures mixed ANOVA).

Conclusions and Clinical Relevance—The findings strongly suggested that use of a weight-adjusted bronchoalveolar lavage technique provided a more uniform ELF recovery, compared with that for a fixed-amount bronchoalveolar lavage technique, when urea was used as a marker of dilution. A constant ELF fraction can facilitate more accurate comparisons of cellular and noncellular constituents in BALF among patients of various sizes. (*Am J Vet Res* 2011;72:694–698)

The central issue in BAL is the recovery of a uniform amount of ELF for analysis of cellular and noncellular constituents. The ELF is a thin layer of fluid that covers the epithelium of the alveoli and small airways. During BAL, ELF dissolves in saline (0.9% NaCl) solution to yield BALF. The determination of total amounts of constituents (eg, cells, proteins, and bacteria) in BALF is affected by variations in ELF dilution. The volume of ELF can be calculated by determining the amount of an endogenous solute that exists naturally in BALF or an exogenous molecule that is added to the lavage fluid and comparing that amount with the plasma

ABBREVIATIONS

BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
ELF	Epithelial lining fluid

concentration or the initial concentration of the exogenous molecule, respectively. However, feasibility for the use of extrinsic molecules (eg, methylene blue, inulin, or radioactive tracers) requires additional measures for both the lavage and analysis phases; therefore, it is easier to use endogenous markers (eg, urea, albumin, protein, or potassium).^{1,2} Urea has been considered to be the most reliable endogenous marker of dilution in healthy and diseased lungs.^{2,3} Compared with the use of albumin and protein, urea has the advantage of a low molecular mass that allows rapid diffusion from plasma into ELF, which results in equal urea concentrations in both the plasma and ELF. Use of urea as a dilutional marker to calculate ELF recovery has been reported in horses,¹ humans,^{2,4} dogs,^{5,6} and cats.⁷

Marked variations in volume of ELF recovered have been described,^{8,9} which makes it difficult to compare results among studies. Factors (including the lavage technique, volume of lavage, and amount of time that

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elapses between fluid instillation and the first attempted aspiration of instilled fluid) that affect the amount of ELF recovered have not been standardized. Studies^{2,9} in humans have revealed that the amount of ELF recovered is mainly dependent on the fluid volume used for the lavage. In 1 study⁴ conducted in healthy children between 3 and 15 years of age, adjustment of BAL volume on the basis of body weight yielded constant fractions of ELF. However, the authors are not aware of any studies conducted to compare weight-adjusted and fixed-amount BAL techniques in dogs. The objective of the study reported here was to assess in healthy Beagles whether a BAL technique adjusted on the basis of body weight would yield a more uniform ELF recovery than would a fixed-amount BAL technique.

Materials and Methods

Dogs—The study group consisted of 13 healthy Beagles (5 females and 8 males) that ranged from 5 to 11 years of age (median, 8 years). Body weight of the dogs ranged from 9 to 20 kg (median, 15 kg). Weight index of each dog was estimated by use of a 5-point body condition scoring system¹⁰ (1 = underweight, 2 = lean, 3 = optimum, 4 = overweight, and 5 = obese). The health status of each dog was assessed before dogs were sedated for BAL; health status was assessed on the basis of results of physical examination, hematologic and serum biochemical analysis, thoracic radiography, and arterial blood gas analysis.^a Feces were examined for parasite eggs and larvae. Dogs were cared for throughout the study in accordance with principles outlined in a National Institutes of Health publication,¹¹ and the study protocol was approved by the Ethics Committee for Animal Experimentation at the University of Helsinki.

BAL—Dogs were sedated by IM administration of medetomidine^b (20 µg/kg) combined with butorphanol tartrate^c (0.1 mg/kg). Anesthesia was induced with propofol^d (1 mg/kg, IV); the same dose of propofol was administered again, if needed. Bronchoscopy was performed with the dogs in sternal recumbency.

Dogs were allocated to 2 groups by use of a random allocation (ie, the names of the dogs were written on pieces of paper, which were then placed in a basket and removed one at a time by an assistant; the dogs were alternately assigned to groups 1 and 2 on the basis of the order in which their names were drawn from the basket). In 1 group (n = 6 dogs), the right caudal lung lobe was lavaged on the basis of each dog's body weight (2 mL/kg, divided into 2 aliquots) and the left caudal lung lobe was lavaged with a fixed amount of fluid (50 mL/dog, divided into 2 aliquots). In the second group (7 dogs), the right caudal lung lobe was lavaged with a fixed amount of fluid (50 mL/dog, divided into 2 aliquots) and the left caudal lung lobe was lavaged on the basis of each dog's body weight (2 mL/kg, divided into 2 aliquots). If a dog's weight index differed from the optimum value of 3, the optimal body weight for that dog was estimated, and the volume of the lavage fluid was adjusted accordingly.

The tip of the bronchoscope^e was first wedged into the right caudal lung lobe. Sterile warm (37°C) sa-

line solution was infused through the biopsy channel, which was followed by infusion of 5 mL of air to empty the channel. Gentle manual aspiration with a 20-mL syringe was applied immediately after infusion of the saline solution. The investigators attempted to achieve the same aspiration pressure for all dogs and aliquots. All BAL procedures were performed by a single investigator (MAM). Aspiration was repeated multiple times, and the syringe was emptied as needed; aspiration procedures were stopped when no more fluid was recovered. Fluid was collected in a glass container placed on ice. The BAL procedure was repeated in the same lung lobe with the second aliquot. The left caudal lung lobe then was lavaged in a similar manner, except that a different volume of fluid was used.

A recovery percentage $\geq 40\%$ of the instilled fluid volume was required. Total duration of BAL for each lung lobe (ie, lavage time elapsed from the beginning of the instillation of the first aliquot until the end of the last aspiration attempt after infusion of the second aliquot) was measured for both groups. Dwell time (time that elapsed between fluid instillation and the first attempted aspiration) was < 30 seconds in all dogs.

Examination of BALF—The BALF specimens from the right and left caudal lung lobes were examined immediately after collection. Quantitative bacterial culture was performed by inoculating a 10-µL sample of unfiltered BALF on a blood-agar plate. The plate was incubated at 37°C for 48 hours, and bacterial growth of $> 1.7 \times 10^3$ CFUs/mL was used as an indicator of bacterial infection.¹²

Urea concentrations in serum and BALF were determined with a kinetic enzymatic method¹³ by use of a clinical chemistry analyzer^f and a commercial reagent.^g The intra-assay and interassay coefficients of variation for the serum urea determination were 2.5% (mean, 48.3 mg/dL; n = 10 observations) and 2.8% (mean, 55.9 mg/dL; 30 observations), respectively. The intra-assay variability of 8 BALF urea assays was 4.5% and 0.9% for concentrations of 0.62 and 2.54 mg/dL, respectively. The observed concentrations of BALF urea relative to expected concentrations after addition of known amounts of urea that ranged from 0.31 to 2.50 mg/dL were 97.6% to 101.5%. The detection limit of the BALF urea assay was 0.08 mg/dL. The proportion amount of ELF was calculated by use of the following equation: (concentration of urea in BALF/concentration of urea in serum) $\times 100\%$.

Statistical analysis—Data were expressed as mean \pm SD or median and range. Parametric analyses were used when normal distribution of data was verified. Statistical software programs^{h-j} were used for the statistical analyses. For all analyses, values of $P < 0.05$ were considered significant.

Comparison of BALF recovery percentages between fixed-amount and weight-adjusted techniques was performed with paired *t* tests. Comparisons of lavage times and BALF total cell counts between techniques were analyzed with Wilcoxon matched-pairs sign rank tests. The association between lavage time and BALF urea concentration was analyzed via the Spearman correlation coefficient.

The SDs of the proportions of ELF for the weight-adjusted and fixed-amount techniques were compared by use of a repeated-measures mixed ANOVA. Unstructured covariance structure (which enables differing SDs) was tested against compound symmetry structure (which requires equal SDs) by use of a general linear models procedure.^k

Results

Dogs—Results of physical examination were unremarkable in all dogs. Weight index was 3 (optimum) in 12 dogs and 4 (overweight) in 1 dog. Results of hematologic and serum biochemical analyses as well as mean \pm SD PaO₂ (97.6 \pm 7.4 mm Hg) and PaO₂-PaO₂ (9.9 \pm 6.6 mm Hg) were within reference ranges in all dogs, with minor exceptions. All fecal analyses for parasites yielded negative results. Thoracic radiography revealed only mild age-related findings.

BAL—Mean \pm SD recovery percentage of infused lavage fluid was 58 \pm 13% for the fixed-amount BAL technique and 57 \pm 11% for the weight-adjusted BAL technique; no significant difference ($P = 0.81$) was detected between the techniques. Total cell counts did not differ significantly ($P = 0.31$) between the fixed-amount (median, 270 cells/ μ L; range, 120 to 730 cells/ μ L) and weight-adjusted (median, 250.0 cells/ μ L; range, 190 to 820 cells/ μ L) BAL techniques. Median differential cell counts for the fixed-amount and weight-adjusted BAL techniques were 74.4% (range, 62.4% to 87.4%) and 78.4% (range, 61.0% to 87.0%), respectively, for macrophages; 19.4% (range, 11.0% to 33.7%) and 15.4% (range, 9.0% to 31.4%), respectively, for lymphocytes; 2.7% (range, 0.7% to 5.4%) and 2.0% (range, 1.4% to 7.0%), respectively, for neutrophils; 1.7% (range, 1.0% to 7.0%) and 2.0% (range, 0.4% to 5.7%), respectively, for mast cells; 0% (range, 0% to 3.4%) and 0.4% (range, 0% to 1.0%), respectively, for eosinophils; 0% (range, 0% to 1.0%) and 0% (range, 0% to 3.7%), respectively, for plasma cells; and 0% (range, 0% to 0%) and 0% (range, 0% to 0%), respectively, for epithelial cells. Bacterial cultures yielded negative results, and no intracellular bacteria were detected. Serum urea concentrations

ranged from 16.5 to 28.6 mg/dL (median, 19.3 mg/dL), and BALF urea concentrations ranged from 0.27 to 1.1 mg/dL (median, 0.53 mg/dL).

Lavage time for 1 lung lobe did not differ significantly ($P = 0.15$) between the fixed-amount (median, 11.3 minutes; range, 9.4 to 18.2 minutes) and weight-adjusted (median, 10.4 minutes; range, 9.1 to 15.1 minutes) BAL techniques. No association between urea concentration in BALF and lavage time was detected for either BAL technique (fixed-amount technique, $r = 0.12$ [$P = 0.69$]; weight-adjusted technique, $r = 0.43$ [$P = 0.19$]).

Mean \pm SD proportion of ELF calculated by use of the urea method was 2.89 \pm 0.89% for the fixed-amount technique and 2.28 \pm 0.39% for the weight-adjusted technique (Figure 1). The SDs differed significantly ($P = 0.041$) between the 2 BAL techniques.

Discussion

Examination of BALF is a method that is useful in the diagnosis and study of alveolar and small airway diseases in dogs. The proportion of ELF recovered in BALF does not affect relative cell counts provided sufficient fluid is infused to avoid collecting samples primarily from the large airways.¹⁴ However, when BALF is used for quantitative assessment of constituents in recovered fluid, fluctuations in ELF recovery may cause marked variation in results; thus, it is vital to collect a uniform amount of ELF in consecutive lavages. Few studies¹⁵⁻¹⁷ have been conducted to solve this problem via development of methods to collect pure ELF, and such techniques are not yet appropriate for routine use. In the present study, we found that adjustment of the volume of lavage fluid on the basis of body weight provides a more uniform recovery of ELF in dogs than does use of a fixed-amount volume of lavage fluid.

In the study reported here, dilution of ELF was determined by use of the urea method, as described elsewhere.² Urea is a good marker of dilution; it is a physiologic molecule with no metabolism in lung cells, has comparable concentrations in various body fluids, and is easy to measure.² The major problem with this method is the possible overestimation of the recovered ELF volume caused by diffusion of urea into ELF during lavage, especially in cases of prolonged dwell time or concomitant lung disease with altered membrane permeability.^{9,18} Despite these factors, the urea method is considered sufficiently reliable provided the aspiration of instilled saline solution is initiated without delay and the dwell time for lavage fluid remains short.¹⁹ In the present study, diffusion of urea was not expected because dwell times were short (ie, < 30 seconds).

Investigators in other studies^{2,9} have suggested that in addition to dwell time, lavage time (ie, duration of BAL) has an effect on urea diffusion. In 1 study,⁹ investigators performed BAL in healthy human volunteers with lavage fluid volumes of 100 and 300 mL and found that the diffusion of urea increased significantly beginning with the third 20-mL or with the 50-mL aliquot when BAL lasted 2.0 and 4.1 minutes, respectively. However, investigators in another study²⁰ in healthy humans found no relationship between influx of urea and duration of BAL when lavage time varied from 2.7

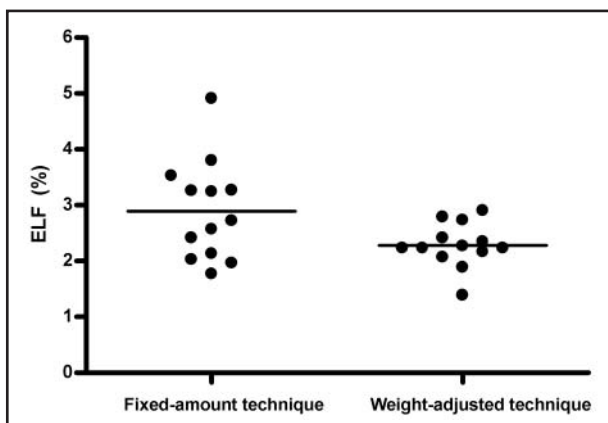


Figure 1—Proportions of ELF recovered by use of fixed-amount and weight-adjusted BAL techniques in 13 dogs. Each symbol represents results for 1 dog, and the horizontal line indicates the mean value for each technique. The SDs differed significantly ($P = 0.041$) between the 2 techniques.

to 7.0 minutes. Although lavage times in the present study were > 7.0 minutes because of efforts to maximize the amount of recovered fluid and to enable us to evaluate the time effect on urea concentration in BALF, we did not find that an increase in BAL duration caused an increase in urea concentration in BALF.

Mean ELF recovery of 2.3% for the weight-adjusted technique and 2.9% for the fixed-amount technique are slightly higher than the recovery percentages (range, 1.0% to 2.1%) reported for dogs in other studies.^{5,6,17} This can be explained by differences in methods among studies, including variations in aspiration technique, aspiration pressure, volume of lavage fluid, number of aliquots, dwell time, BAL duration, and preparation of BALF sample. However, the key issue in the study reported here is that the variability in the proportion of recovered ELF described by the SDs was smaller for the weight-adjusted technique than for the fixed-amount technique. Therefore, we believe that the accuracy for analyses of constituent concentrations in BALF is better for the weight-adjusted technique and that the estimate of absolute amounts of constituents in ELF is more exact.

Healthy dogs were used in the study reported here. It has been speculated that lung disease can change the permeability of the alveolar-capillary membrane and allow additional influx of urea into BALF, thus complicating the use of urea as a marker of dilution.²⁰ In contrast, investigators in another study³ compared various markers of dilution in infants with and without lung disease and concluded that urea is a more reliable marker of dilution than is protein, albumin, sphingomyelin, or IgA secretory component. In addition, that study³ revealed no evidence of additional influx of urea into the lavage fluid in association with epithelial disruption in diseased lungs. Significant variations in albumin and protein concentrations in ELF have been detected among diseased, recovering, and healthy lungs.^{3,21,22} Additionally, elevated albumin concentrations in ELF have been associated with increased age in humans.²³ The use of urea and inulin as dilutional markers has been evaluated in healthy horses and horses with chronic obstructive pulmonary disease (ie, heaves).¹ In that study,¹ investigators found that ELF recovery was significantly higher when calculated via the inulin method and there was no correlation between the ELF percentages calculated with inulin or urea. However, they concluded that combined use of both markers may yield an advantage by providing upper and lower limits of ELF recovery. If the study reported here were to be repeated in dogs with lung disease, diffusion of urea might be altered and use of additional endogenous or extrinsic markers of dilution would be needed to verify accuracy of the urea method.

The use of dogs with a broader range of body weights (ie, from small-breed dogs to giant-breed dogs) would have further elucidated the effect of adjustment of the volume of lavage fluid on the basis of body weight. Although the study population had only moderate variation for size, the result of a more uniform ELF recovery is consistent with the findings in a study⁴ in children between 3 and 15 years of age with broad differences in weights. Because airways grow in paral-

lel with overall body size, adjustment of the volume of lavage fluid on the basis of body weight appears to be justified.²⁴

We concluded that when the aim of BALF analysis is to measure exact amounts of constituents (eg, bacteria and proteins) in ELF for comparison of results, recovery of a uniform ELF volume is essential. Analysis of our results revealed that in healthy Beagles, the use of a volume of lavage fluid adjusted on the basis of body weight is 1 method for a more uniform ELF recovery.

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- a. ABL 800 FLEX, Radiometer A/S, Brørshøj, Denmark.
 - b. Domitor, Orion Oyj, Espoo, Finland.
 - c. Torbugesic, Scanvet Animal Health A/S, Fredensberg, Denmark.
 - d. Rapinivet, Schering-Plough A/S, Ballerup, Denmark.
 - e. Olympus GIP type N30, Olympus Optical Co Ltd, Tokyo, Japan.
 - f. Kone Specific, ThermoFisher Scientific, Vantaa, Finland.
 - g. UREA UV 250, BioMerieux SA, Marcy-l'Étoile, France.
 - h. GraphPad Prism, version 4.01, GraphPad Software Inc, La Jolla, Calif.
 - i. Statistix 9.0 for Windows, Analytical Software, Tallahassee, Fla.
 - j. SAS, version 9.1, SAS Institute Inc, Cary, NC.
 - k. PROC GENMOD, SAS, version 9.1, SAS Institute Inc, Cary, NC.
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