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

Marika A. Melamies, DVM; Anna-Kaisa Jarvinen, DVM, PhD; Kati M. Seppälä, DVM; Hannu J. Rita, PhD; Minna M. Rajamäki, DVM, PhD

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 ≥ 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) X 100.

Results—Mean ± SD proportion of ELF in BALF was 2.28 ± 0.39% for the weight-adjusted technique and 2.89 ± 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)
elapses between fluid instillation and the first attempt-
ed aspiration of instilled fluid) that affect the amount of
ELF recovered have not been standardized. Studies in humans have revealed that the amount of ELF re-
covered is mainly dependent on the fluid volume used for
the lavage. In 1 study conducted in healthy chil-
dren between 3 and 15 years of age, adjustment of BAL
volume on the basis of body weight yielded constant
fractures 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 system (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 ba-

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 investi-
gator (MAM). Aspiration was repeated multiple times,
and the syringe was emptied as needed; aspiration pro-
cedures were stopped when no more fluid was recov-
ered. 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 dif-
ferent volume of fluid was used.

A recovery percentage ≥ 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 ali-
quots) was measured for both groups. Dwell time (time
that elapsed between fluid instillation and the first at-
tempted aspiration) was < 30 seconds in all dogs.

Examination of BALF—The BALF specimens from
the right and left caudal lung lobes were examined im-
mediately after collection. Quantitative bacterial cul-
ture 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 × 10^3 CFUs/mL was used as an indicator of bacte-
rial infection.12

Urea concentrations in serum and BALF were de-
termined with a kinetic enzymatic method by use of a
clinical chemistry analyzer and a commercial reagent.8
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 4.3% and 0.9%
for concentrations of 0.62 and 2.54 mg/dL, respective-
ly. 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) × 100%.

Statistical analysis—Data were expressed as mean
± SD or median and range. Parametric analyses were
used when normal distribution of data was verified.
Statistical software programs were used for the statisti-
cal 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 per-
cformed 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 correla-
tion 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.5

**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 ± SD PaO₂ (97.6 ± 7.4 mm Hg) and PaO₂–PaCO₂ (9.9 ± 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 ± SD recovery percentage of infused lavage fluid was 58 ± 13% for the fixed-amount BAL technique and 57 ± 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/µL; range, 120 to 730 cells/µL) and weight-adjusted (median, 250.0 cells/µL; range, 190 to 820 cells/µL) 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 ± SD proportion of ELF calculated by use of the urea method was 2.89 ± 0.89% for the fixed-amount technique and 2.28 ± 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.5 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.14 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, dilution of urea was not expected because dwell times were short (ie, < 30 seconds).

Investigators in other studies13–19 have suggested that in addition to dwell time, lavage time (ie, duration of BAL) has an effect on urea diffusion. In 1 study,17 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 2.0-4.1 minutes and weighed. 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...
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.\textsuperscript{3,5,17} 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.\textsuperscript{20} In contrast, investigators in another study\textsuperscript{3} 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 study\textsuperscript{3} 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.\textsuperscript{1,2,11,22} Additionally, elevated albumin concentrations in ELF have been associated with increased age in humans.\textsuperscript{23} The use of urea and inulin as dilutional markers has been evaluated in healthy horses and horses with chronic obstructive pulmonary disease (ie, horses),\textsuperscript{1} In that study,\textsuperscript{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 study\textsuperscript{4} in children between 3 and 15 years of age with broad differences in weights. Because airways grow in parallel with overall body size, adjustment of the volume of lavage fluid on the basis of body weight appears to be justified.\textsuperscript{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.

confirmed that the use of dogs with a broader range of body weight appears to be justified.24


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