CYTOLOGIC ANALYSIS OF BRONCHOALVEOLAR LAVAGE FLUID COLLECTED THROUGH AN ENDOTRACHEAL TUBE IN DOGS*

MARCELA FREIRE VALLIM DE MELLO1, ANA MARIA REIS FERREIRA2 & AMARY NASCIMENTO JR.3

Abstract

Bronchoalveolar lavage is a procedure that retrieves cells and other elements from the lungs for evaluation, and helps in the diagnosis of many pulmonary diseases. The aims of this study were: to perform this procedure in dogs, using an endotracheal tube, to analyse the percentage of fluid recovered and to evaluate cell preservation in the specimens. Bronchoalveolar lavage was performed in 27 dogs, through an endotracheal tube, for cytologic analysis. The dogs were positioned either in dorsal or right lateral recumbency and the percentages of fluid recovered were statistically compared. Cell preservation was evaluated in specimens which were processed at different times. The average percentage of the collected fluid in 19 dogs positioned in dorsal recumbency was 22.66 ± 13.21% and it was 16.84 ± 13.78% in 8 dogs positioned in right lateral recumbency. There was no significant difference (P>0.05) between the percentages of collected fluid in the two types of recumbency. The smears prepared within 24 hours after collection showed a significant decrease (P<0.05) in the percentage of neutrophils and eosinophils and a consequent increase in the percentage of macrophages, when compared to smears prepared within 3 hours after lavage. These results indicated that both types of recumbency provide satisfactory collection of specimens, in terms of quantity, and that the correct diagnosis of inflammatory diseases depends on quickness in smear preparation. The use of an endotracheal tube was considered efficient for collecting specimens from deep portions of the lungs in dogs.

Key words: bronchoalveolar lavage, cytology, dog.
INTRODUCTION

Bronchoalveolar lavage (BAL) is a procedure performed in dogs to collect representative samples from the lungs. It helps the diagnosis of many pulmonary diseases [7,10-12]. This technique retrieves cells and other elements that line the epithelial surfaces of the small airways and alveoli for cytologic evaluation, providing information about inflammatory and neoplastic diseases [2,3,6,15,22,29].

Different methods of bronchoalveolar lavage have been described in veterinary reports [8-11,16-18,20]. In dogs and cats, flexible fiberoptic bronchoscopes are often used for the collection of BAL fluid [11,23,28,29]. Nevertheless, the introduction of a bronchoscope in very small pets may be impossible due to the small diameter of trachea and bronchi [9,11].

The aims of this study were: to perform BAL in dogs, using an endotracheal tube, to compare the percentage of fluid recovered between dorsal and right lateral recumbency and to evaluate cell preservation in specimens which were processed at different times after collection.

MATERIALS AND METHODS

Twenty-seven young dogs from indefinite breeds were obtained from the Center of Zoonosis Control situated in Niteroi, Rio de Janeiro, Brazil. Fifteen dogs were males and twelve were females and their body weight ranged from 5 to 23 kg. All dogs underwent physical examinations and investigative procedures, including complete blood counts and serum biochemical profiles.

Anesthesia was induced with sodium thiopental (Thionembutal) and maintained with halothane (Halothano) or additional sodium thiopental, when necessary. 100% oxygen was administered to the dogs for 10 minutes before the lavage procedure.

Nineteen dogs were positioned in dorsal recumbency and eight in right lateral recumbency. Bronchoalveolar lavage was performed through an endotracheal tube (diameter 5.5 to 8.5 mm), which was placed to be rostral to the carina. The cuff on the endotracheal tube was expanded and a urinary catheter (Sovereign polypropylene urethral catheter 8 Fr. X 22") was inserted into the tube to the level of the carina. The catheter was modified to give a terminal “eye”, rather than a lateral opening, to allow the lavage fluid to flow and to return more easily.

Two 5 ml/kg aliquots of sterile saline solution (Solução Fisiológica Cloruro de Sódio 0.9%) were gently injected through the catheter by sterile syringe. Immediately after each injection, mild suction was applied to the syringe to retrieve the BAL fluid. The dogs received 100% oxygen through the endotracheal tube for 10 minutes after the lavage procedure.

The two aliquots of collected fluid were combined and had their volumes measured. All the samples were maintained under refrigeration until the smear preparation. Mucus was collected from the samples with a pipette and transferred to glass microscope slides for the preparation of direct smears. The fluid part of the sample was processed in a cytocentrifuge. Four hundred microliters of the fluid were used to prepare each smear by cytocentrifugation at 1000 rpm for 5 minutes. Each sample was stained with both Wright’s and Papanicolaou’s stains [1].

All the samples were processed within three hours after lavage. In order to evaluate the cell preservation in saline solution, 9 of the 27 cases studied had part of the BAL fluid reserved in refrigeration for cytocentrifuged smear preparation 24 hours after the procedure.

The cytologic analysis included qualitative cellular evaluation and a differential cell count of 200 cells per slide. The cell count was reported as relative count (percentage of total white blood cells). The cell types considered in this calculation were macrophages, lymphocytes, neutrophils and eosinophils. The differential cell count was only performed on cytocentrifuged smears stained with Wright’s stain.

The fluid recovered was statistically compared between the animals positioned in dorsal and right lateral recumbency, using the Wilcoxon-Mann-Whitney test for independent samples. The results of cell counts performed on smears prepared in different times after collection (3 and 24 hours) were compared using the Wilcoxon test for paired samples. All differences between the groups were considered significant when P values were below 0.05.
RESULTS

The clinical signs observed in dogs after BAL consisted of cough and moist rales during auscultation. The clinical signs resolved spontaneously within 24 hours after the lavage procedure.

The collected fluid had a foamy appearance in 88% of the cases studied and cloudy appearance in 33% of the cases. Small threads of mucus were present in 92% of the collected specimens.

The average percentage of injected fluid that was recovered in all dogs was 20.94 ± 13.39% (range, 3.18 to 60.0%). The average percentage in 19 dogs positioned in dorsal recumbency was 22.66 ± 13.21% (range, 3.18 to 60.0%) and it was 16.84 ± 13.78% (range, 4.66 to 42.5%) in 8 dogs positioned in right lateral recumbency. There was no significant difference (P<0.05) between the percentage of injected fluid recovered in the two types of recumbency.

The bronchoalveolar lavage fluid cytologic analysis revealed alveolar macrophages, lymphocytes, neutrophils, eosinophils, ciliated and non-ciliated columnar and cuboidal epithelial cells (Fig.1). Epithelial cells were found in small quantity, individually or in clusters. Erythrocytes were present in 12 specimens. Bacterial rods were scattered throughout the slide in one case (Fig.1-D) and rare mast cells were seen in another case. Vacuolated macrophages with foamy cytoplasm and phagocytized particles were found in some cases (Fig.1-B). Binucleated macrophages and multinucleated giant cells were rarely found (Fig.1-C).

The differential cell count revealed a great diversity of relative values among the 27 cases studied (Tab.1). Eleven of the dogs had cell relative numbers within the BAL cell reference values reported from clinically healthy dogs [10,11,24-28]. Five of the dogs examined had an increased percentage of neutrophils. Cytologic analysis showed high numbers of bacterial rods in one of these cases and hemorrhage in another one (Fig.1-D,E). Two dogs had high percentage of lymphocytes and nine dogs had increased percentage of eosinophils.

The differential cell count revealed a significant decrease (P<0.05) in the number of neutrophils and eosinophils and a consequent increase in the number of macrophages, when comparing the smears prepared 24 hours after the BAL and the ones prepared within 3 hours after the procedure (Tab.2). Nevertheless, the cells did not show any morphologic alteration.

The main cell type present in cytocentrifuged smears was the same as the one present in direct smears prepared with mucus in 16 cases (59%; Fig.1-F).

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Values within the normal reference range* (11 dogs)</th>
<th>Acute inflammation (5 dogs)</th>
<th>Immunologic reaction (2 dogs)</th>
<th>Hypersensitivity reaction (9 dogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages</td>
<td>66.9 ± 13.5 (43.0 - 88.0)</td>
<td>13.0 ± 6.3 (7.0 - 23.0)</td>
<td>30.0 ± 5.6 (26.0 - 34.0)</td>
<td>48.3 ± 12.4 (23.0 - 67.0)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>19.4 ± 12.9 (4.5 - 43.5)</td>
<td>1.7 ± 1.3 (0 - 3.5)</td>
<td>63.2 ± 13.0 (54.0 - 72.5)</td>
<td>13.2 ± 7.1 (5.0 - 23.0)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3.9 ± 4.2 (0 - 11.5)</td>
<td>79.1 ± 11.2 (60.5 - 88.5)</td>
<td>1.75 ± 1.0 (1.0 - 2.5)</td>
<td>7.6 ± 9.9 (0.5 - 31.0)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>9.7 ± 4.8 (0 - 14.0)</td>
<td>6.0 ± 5.3 (0 - 14)</td>
<td>5.0 ± 6.3 (0.5 - 9.5)</td>
<td>30.8 ± 8.0 (17.5 - 44.5)</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation and range of cell percentage found in each group of dogs. *From Hawkins & Denicola [10], Hawkins et al. [11], Pinsker et al. [24], Rajamaki et al. [25], Rebar et al. [26], Sommerhoff et al. [27], Vail et al. [28].

**Figure 1.** A- Cytocentrifuged preparation of bronchoalveolar lavage fluid from a dog (case 27). Two eosinophils are present in the center, surrounded by three lymphocytes. The largest cells are alveolar macrophages. Wright’s stain. Bar = 10mm. B- Cytocentrifuged preparation from the same sample as figure 1. Two vacuolated alveolar macrophages are present in the upper left center. There are two neutrophils with segmented nucleus, one of which is in the lower center and contains two phagocytized erythrocytes. The small round cells are lymphocytes. Papanicolaou’s stain. Bar = 10mm. C- Cytocentrifuged preparation of bronchoalveolar lavage fluid from a dog (case 6). Multinucleated giant cell surrounded by alveolar macrophages. Papanicolaou’s stain. Bar = 10mm. D- Cytocentrifuged preparation of bronchoalveolar lavage fluid from a dog with acute inflammation (case 3). The field contains an alveolar macrophage with multiple aggregates resembling coal dust, ciliated epithelial cells and bacterial rods. Wright’s stain. Bar = 10mm. E- Cytocentrifuged preparation of bronchoalveolar lavage fluid from a dog with acute inflammation (case 15). The predominant cells are neutrophils and erythrocytes. The largest cells are alveolar macrophages. Papanicolaou’s stain. Bar = 20mm. F- Direct smear of bronchoalveolar lavage fluid from a dog with acute inflammation (case 12). Numerous neutrophils and alveolar macrophages are adhered to strands of mucus. The same cellular pattern was observed in the cytocentrifuged preparation of this sample. Papanicolaou’s stain. Bar = 20mm.
DISCUSSION

The clinical signs observed in dogs after BAL were attributed to the injection of a relatively high volume of fluid into the lungs. Nevertheless, the signs resolved within 24 hours after the lavage procedure, without the need of therapeutic assistance, as previously reported [20,21].

The foamy appearance of the collected fluid in 88% of the lavage procedures performed in this study reflected the presence of surfactant. Hawkins & Denicola [9] and Hawkins et al. [11] suggested that the presence of surfactant in BAL fluid indicates the lavage of the deep portions of the lungs and the collection of representative cells from the small airways and alveoli. The cloudy appearance observed in BAL fluid in 33% of the cases studied was associated with high cellularity. The presence of small threads of mucus in 92% of the cases had no clinical significance, once it is a common element of the airways, produced by the goblet cells.

The average percentage of collected fluid was relatively low in either dorsal or right lateral recumbency (22.66 and 16.84%, respectively), compared to the average volumes obtained in previous reports [9,13,20]. However, the dogs in the present study were not tilted to a head-down position, in order to assist the reflof of the fluid, as it has been done in those reports. And even the smallest volumes collected provided adequate specimens for cytologic evaluation.

The absence of a significant difference (P>0.05) in fluid reflof between the dorsal and right lateral recumbency results suggested that both types of recumbency can provide satisfactory collection of specimens, in terms of quantity.

The bronchoalveolar lavage fluid is characterized by a great diversity of cells like alveolar macrophages, lymphocytes, neutrophils, eosinophils, mast cells and a small quantity of epithelial cells [11,13]. The predominance of epithelial cells in collected specimens represents the lavage of large airways instead of BAL [4,13]. The cell types collected in association with surfactant in the present study, indicated that the BAL technique used in the dogs, allowed the collection of representative specimens from the deep portions of the lungs.

The differential cell count from 16 dogs suggested pulmonary alterations. Acute neutrophilic inflammation was suggested in 5 dogs, which presented 60.5 to 88.5% of neutrophils in BAL fluid. Bronchoalveolar lavage fluid cytologic analysis of one of these cases revealed high number of bacterial rods, suggesting bacterial infection. Another case also presented hemorrhage. Immunologic reaction was suggested in 2 cases, due to high numbers of lymphocytes in the BAL fluid. Hypersensitivity reaction was sug
gested in 9 dogs that showed a high percentage of eosinophils.

The alterations in cellular morphology described in previous reports [5,26] were not observed in specimens processed 24 hours after collection. However, the significant decrease (P<0.05) in neutrophil and eosinophil numbers in the specimens, when compared to specimens processed within three hours after lavage indicated the importance of smear preparation as soon as possible after BAL. The reason for the instability of neutrophils and eosinophils in samples stored in refrigeration for 24 hours is not clear and it requires additional research.

The correspondence in the frequency of cell types observed, in direct smears prepared with mucus and the ones in cytocentrifuged smears in 59% of the cases studied, suggested the importance of evaluating the cells adhered to mucus. This result reveals that epithelial cells from the airways are not always the predominant cell type adhered to mucus, as previously described [14] and that leukocytes, found in direct smears prepared with mucus, can be representative of the deep portions of the lungs.

The bronchoalveolar lavage through an endotracheal tube, using two aliquots of 5 mL/kg of saline solution, was considered a quick and efficient method of collecting specimens in dogs. The clinical signs observed in the animals after BAL were not significant enough to disregard the use of the technique described in this paper. However, due to the relatively high volume injected into the lungs, this lavage procedure must only be performed on stable patients which are free from respiratory distress.

CONCLUSIONS

The bronchoalveolar lavage fluid cytologic evaluation can provide valuable information about pulmonary diseases, once the collected specimens are of high quality and represent the deep portions of the lungs [11]. The collected specimens can indicate the presence of focal and diffuse pulmonary changes, narrowing the list of differential diagnoses or even providing the definitive diagnosis [19]. This simple examination should be recommended for frequent use in the small animal clinical practice.

REFERENCES


SOURCES AND MANUFACTURES

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4 Darrow Laboratórios S/A, RJ, Brasil.


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