

Bronchoalveolar Lavage Fluid and Serum Canine Surfactant Protein A Concentrations in Dogs with Chronic Cough by Bronchial and Interstitial Lung Diseases

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ABSTRACT. We measured bronchoalveolar lavage fluid (BALF) and serum canine surfactant protein (cSP)-A concentrations in dogs with chronic cough. There were no significant differences between bronchial and interstitial lung diseases in BALF cSP-A concentrations. However, serum cSP-A concentrations in dogs with the interstitial lung disease as diffuse panbronchiolitis and idiopathic pulmonary fibrosis were significantly higher than those in dogs with the bronchial disease as chronic bronchitis. These results suggest that serum cSP-A concentrations may be a useful and noninvasive biomarker to understand the existence of interstitial lung damage in dogs with chronic cough.

KEY WORDS: canine, chronic cough, SP-A.

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It is well known that the two roles of pulmonary surfactant protein (SP) include the reduction of surface tension at the air liquid interface in alveoli [4, 18] and the regulation of immunological defense mechanisms in the lung [2]. The latter is particularly mediated by SP-A and SP-D, which are two of four subgroups of pulmonary SP [25]. Pulmonary SP is mainly synthesized and secreted from type II cells and airway Clara cells [24], and it is considered that SP-A leaks into the blood stream after lung injuries in human [10].

In Japan, serum SP-A concentrations in humans are clinically useful as a diagnostic biomarker of respiratory diseases [11]. In dogs, the aspect of cSP-A has been known for a long time [12]; however, there are no methods to measure cSP-A concentrations. Recently, it has been reported that methods to measure cSP-A concentrations using sandwich ELISA have been developed [20] and that the serum cSP-A concentrations in dogs with aspiration pneumonia, primary lung tumors and blunt traumatic lung injury are higher than those in healthy dogs [21]. The cause of this increase in serum cSP-A concentration is considered to be an increase in SP-A secretions at the lung lesion or leakage from lung lesions into the bloodstream. Therefore, the elevation ratio of serum cSP-A concentration in each respiratory disease may be different,

but this is unknown in dogs. In this study, bronchoalveolar lavage fluid (BALF) and serum cSP-A concentrations were measured whether types of respiratory diseases in dogs with chronic cough can be classified by these concentrations.

A total of 19 BALF samples from right middle, accessory or left caudal part of cranial lobe and 19 serum samples from 19 dogs were examined. These dogs were referred to the Animal Medical Center of Nihon University (2005.4–2010.3) to diagnose and consult with regard to long-term therapy for the clinical signs of chronic cough lasting for at least two months. These breeds were miniature dachshund (N=12), Pembroke Welsh Corgi (N=3), Shih Tzu (N=2), Border collie (N=1) and Shetland sheepdog (N=1). BALF samples were gently aspirated through a biopsy channel of the bronchoscope after infusing with sterile saline (0.9% NaCl) solution (40 ml, divided in 2 aliquots) under general anesthesia with isoflurane, and at the same time, 1 ml of whole blood samples was collected from the jugular vein. Serum was then retrieved from the blood samples. All samples were immediately stored in the refrigerator at –20°C until analysis. In addition, the dogs were diagnosed chronic bronchitis (N=8, 6.3 ± 2.4 years old, CB group), diffuse panbronchiolitis (N=6, 7.0 ± 2.6 years old, DPB group) and idiopathic pulmonary fibrosis (N=5, 7.2 ± 3.0 years old, IPF group) by lung patterns on chest CT images (Fig. 1) and bronchoscopic examination, and cardiac diseases were ruled out.

BALF and serum cSP-A concentrations were measured in a manner similar to that of ELISA, which was previously reported [20]. Two different capture and detection antibodies, mouse anti-canine SP-A monoclonal antibodies (MAB3272 and MAB3274, CHEMICON International, Inc., Temecula, CA, U.S.A.) and a Protein Detector ELISA Kit (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, U.S.A.) for

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Fig. 1. Examples of three lung patterns on CT imaging. A: bronchial pattern in chronic bronchitis; B: interstitial pattern by diffuse granular shadows in diffuse panbronchiolitis; C: interstitial pattern by ground glass opacity with septal thickening in idiopathic pulmonary fibrosis.

a two-site sandwich ELISA were used. A standard curve was prepared using purified cSP-A [8, 13] from stocked BALF samples of clinically healthy dogs, which were used in a previous study [22]. In preliminary examination, this assay showed the detection limited of 3.1 ng/ml and the coefficient of variation of 1.28–8.92%. Furthermore, BALF and serum cSP-A concentrations in healthy adult dogs were $2,033 \pm 1,214$ ng/ml and 23 ± 13 ng/ml. In addition, BALF cSP-A concentrations were corrected by urea concentrations (QuantiChrom™ Urea Assay Kit, BioAssay Systems, Hayward, CA, U.S.A.) in serum and BALF [17].

Data obtained from the groups were analyzed for normality by the Shapiro–Wilks test and equal variance by the *F*-test. Mean and standard deviation (SD) or median with minimum and maximum values was used to describe parametric and nonparametric continuous variables, respectively. More than two groups of continuous variables were compared by one-way analysis of variance or Kruskal–Wallis test. If $P < 0.05$, the unpaired *t*-test or Mann–Whitney rank sum test was used in pairwise comparisons to determine which groups were significantly different. $P < 0.05$ was considered significant. All statistical analyses were performed with a statistical software package (SigmaPlot for Windows Version 12.0, SYSTAT SOFTWARE, INC, San Jose, CA, U.S.A.).

BALF cSP-A concentrations were $3,636 \pm 1,632$ ng/ml in CB, $1,585 \pm 988$ in DPB and $2,897 \pm 2,119$ in IPF groups. There were no significant differences among three groups in BALF cSP-A concentrations and the concentrations corrected by urea concentrations ($139,038 \pm 80,819$ ng/ml in CB, $76,293 \pm 84,685$ in DPB and $99,667 \pm 120,606$ in IPF groups). Serum cSP-A concentrations were 45 (22–59) ng/ml in CB, 224 (69–696) ng/ml in DPB and 91 (61–135) ng/ml in IPF groups. There were significant differences among three groups ($P < 0.001$), and serum cSP-A concentrations in both of DPB and IPF groups were significantly higher than those in CB ($P \leq 0.05$) (Fig. 2).

It is challenging to diagnose between respiratory or cardiac disease as primary reasons of chronic cough in dogs. The biomarkers of cardiac diseases, such as ANP, BNP, NT-proBNP and cBNP, have been developed. These increase and are higher in dogs with severe cardiac disease than in

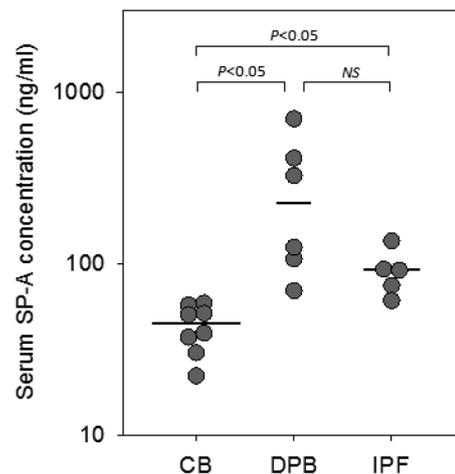


Fig. 2. Plots of serum SP-A concentrations in dogs with chronic cough. Horizontal bars: median, CB: chronic bronchitis, DPB: diffuse panbronchiolitis and IPF: idiopathic pulmonary fibrosis. $P < 0.05$: significant and NS: not significant difference between two groups.

those with respiratory disease and chronic cough [1, 3, 5, 15] and can now be commercially measured during routine clinical practice. In contrast, no clinically useful and specific biomarkers for lung injuries in veterinary medicine are available.

In the lung, pulmonary SP-A enhances pathogen clearance and regulates adaptive and innate immune cell functions [25]. In rats, for example, BALF SP-A concentrations decrease in bacterial pneumonia to detect the pathogen and increase in *Pneumocystis carinii* pneumonia for an immune response to specific pathogens [19]. In horses, BALF SP-A concentrations decrease after prolonged transportation, and this downregulation of lung host defense is considered as one of the mechanisms of transportation-induced fever [7]. In humans with IPF, BALF SP-A concentrations decrease, and the survival time of patients with lower BALF SP-A

concentrations is short [16]. In contrast, serum SP-A concentrations are higher in patients with IPF [14] and reflect the disease activity of IPF [9]. In addition, serum SP-A concentrations in patients with other interstitial lung diseases, radiation pneumonitis [23] and acute respiratory distress syndrome [6] are higher than those in healthy individuals. In this study, dogs with two types of interstitial lung diseases, DPB and IPF, had the higher serum cSP-A concentrations, and the serum cSP-A concentrations in four dogs with CB were below 49 ng/ml, the maximum range (mean + 2SD) in those of healthy dogs. Therefore, we considered that the level of SP-A secretions at the lung lesion is similar in bronchial and interstitial lung diseases but the leakage from lung lesions into the bloodstream increases in interstitial lung diseases, and the damaged bronchioles and alveolar walls are probably essential for detecting the increased serum cSP-A concentration.

Our study had several limitations. First, this study is unable to assess the progress of interstitial lung disease, because the lung lobe that BALF was sampled was usually damaged on all of lung fields in DPB and IPF. However, the CT values of lung field in IPF were likely higher than those in DPB. Second, our cases were limited to mild to moderate respiratory diseases, because of the need for bronchoscopy.

In summary, serum cSP-A concentration may be a useful and noninvasive biomarker to indicate the damage of the lung interstitium in dogs with chronic cough.

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REFERENCES

- Asano, K., Masuda, K., Okumura, M., Kadosawa, T. and Fujinaga, T. 1999. Plasma atrial and brain natriuretic peptide levels in dogs with congestive heart failure. *J. Vet. Med. Sci.* **61**: 523–529. [Medline] [CrossRef]
- Awasthi, S. 2010. Surfactant protein (SP)-A and SP-D as antimicrobial and immunotherapeutic agents. *Recent Pat. Antiinfect. Drug Discov.* **5**: 115–123. [Medline] [CrossRef]
- Boswood, A., Dukes-McEwan, J., Loureiro, J., James, R. A., Martin, M., Stafford-Johnson, M., Smith, P., Little, C. and Attree, S. 2008. The diagnostic accuracy of different natriuretic peptides in the investigation of canine cardiac disease. *J. Small Anim. Pract.* **49**: 26–32. [Medline]
- Clements, J. A. 1957. Surface tension of lung extracts. *Proc. Soc. Exp. Biol. Med.* **95**: 170–172. [Medline] [CrossRef]
- DeFrancesco, T.C., Rush, J.E., Rozanski, E.A., Hansen, B.D., Keene, B.W., Moore, D.T. and Atkins, C.E. 2007. Prospective clinical evaluation of an ELISA B-type natriuretic peptide assay in the diagnosis of congestive heart failure in dogs presenting with cough or dyspnea. *J. Vet. Intern. Med.* **21**: 243–250. [Medline] [CrossRef]
- Greene, K. E., Wright, J. R., Steinberg, K. P., Ruzinski, J. T., Caldwell, E., Wong, W. B., Hull, W., Whitsett, J. A., Akino, T., Kuroki, Y., Nagae, H., Hudson, L. D. and Martin, T. R. 1999. Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. *Am. J. Respir. Crit. Care Med.* **160**: 1843–1850. [Medline] [CrossRef]
- Hobo, S., Yoshihara, T., Oikawa, M. and Jones, J. H. 2001. Surfactant proteins in bronchoalveolar lavage fluid of horses: assay technique and changes following road transport. *Vet. Rec.* **148**: 74–80. [Medline] [CrossRef]
- Hobo, S., Ogasawara, Y., Kuroki, Y., Akino, T. and Yoshihara, T. 1999. Purification and biochemical characterization of pulmonary surfactant protein A of horses. *Am. J. Vet. Res.* **60**: 169–173. [Medline]
- Honda, Y., Kuroki, Y., Shijubo, N., Fujishima, T., Takahashi, H., Hosoda, K., Akino, T. and Abe, S. 1995. Aberrant appearance of lung surfactant protein A in sera of patients with idiopathic pulmonary fibrosis and its clinical significance. *Respiration* **62**: 64–69. [Medline] [CrossRef]
- Ishida, K., Zhu, B., Quan, L., Fujita, M. Q. and Maeda, H. 2000. Pulmonary surfactant-associated protein A levels in cadaveric sera with reference to the cause of death. *Forensic Sci. Int.* **109**: 125–133. [Medline] [CrossRef]
- Kambe, M., Ohshimo, S. and Kohno, N. 2007. Immunity tests for respiratory diseases—SP-A, SP-D, KL-6. *Rinsho Byori* **55**: 381–387 (in Japanese). [Medline]
- King, R. J., Simon, D. and Horowitz, P. M. 1989. Aspects of secondary and quaternary structure of surfactant protein A from canine lung. *Biochim. Biophys. Acta* **1001**: 294–301. [Medline] [CrossRef]
- Kuroki, Y. and Akino, T. 1991. Pulmonary surfactant protein A (SP-A) specifically binds dipalmitoylphosphatidylcholine. *J. Biol. Chem.* **266**: 3068–3073. [Medline]
- Kuroki, Y., Tsutahara, S., Shijubo, N., Takahashi, H., Shiratori, M., Hattori, A., Honda, Y., Abe, S. and Akino, T. 1993. Elevated levels of lung surfactant protein A in sera from patients with idiopathic pulmonary fibrosis and pulmonary alveolar proteinosis. *Am. Rev. Respir. Dis.* **147**: 723–729. [Medline] [CrossRef]
- Lee, J. A., Herndon, W. E. and Rishniw, M. 2011. The effect of noncardiac disease on plasma brain natriuretic peptide concentration in dogs. *J. Vet. Emerg. Crit. Care (San Antonio)* **21**: 5–12. [Medline] [CrossRef]
- McCormack, F. X., King, T. E. Jr., Bucher, B. L., Nielsen, L. and Mason, R. J. 1995. Surfactant protein A predicts survival in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* **152**: 751–759. [Medline] [CrossRef]
- Mills, P. C. and Litster, A. L. 2005. Using urea dilution to standardise components of pleural and bronchoalveolar lavage fluids in the dog. *N. Z. Vet. J.* **53**: 423–428. [Medline] [CrossRef]
- Pattle, R. E. 1955. Properties, function and origin of the alveolar lining layer. *Nature* **175**: 1125–1126. [Medline] [CrossRef]
- Qu, J., He, L., Rong, Z., Pan, J., Chen, X., Morrison, D. C. and Li, X. 2001. Alteration of surfactant proteins A and D in bronchoalveolar lavage fluid of *Pneumocystis carinii* pneumonia. *Chin. Med. J.* **114**: 1143–1146. [Medline]
- Sone, K., Akiyoshi, H., Aoki, M., Sugii, S. and Ohashi, F. 2011. Development and validation of a sandwich ELISA for use in measuring concentrations of canine surfactant protein A in serum of dogs. *Am. J. Vet. Res.* **72**: 833–837. [Medline] [CrossRef]

21. Sone, K., Akiyoshi, H., Shimizu, J., Cao, Z., Li, Y., Tanaka, T., Hayashi, A., Sugii, S. and Ohashi, F. 2013. Surfactant Protein-A Concentration in Sera from Dogs with Pulmonary Parenchymal Diseases. *J. Vet. Med. Sci.* **75**: 685–691. [[Medline](#)] [[CrossRef](#)]
22. Suzuki, K., Yamaya, Y., Kanzawa, N., Chiba, M., Sera, K. and Asano, R. 2008. Trace and major elements status in bronchoalveolar lavage fluid in dogs with or without bronchopneumonia. *Biol. Trace Elem. Res.* **124**: 92–96. [[Medline](#)] [[CrossRef](#)]
23. Takahashi, H., Imai, Y., Fujishima, T., Shiratori, M., Murakami, S., Chiba, H., Kon, H., Kuroki, Y. and Abe, S. 2001. Diagnostic significance of surfactant proteins A and D in sera from patients with radiation pneumonitis. *Eur. Respir. J.* **17**: 481–487. [[Medline](#)] [[CrossRef](#)]
24. Walker, S. R., Williams, M. C. and Benson, B. 1986. Immunocytochemical localization of the major surfactant apoproteins in type II cells, Clara cells, and alveolar macrophages of rat lungs. *J. Histochem. Cytochem.* **34**: 1137–1148. [[Medline](#)] [[CrossRef](#)]
25. Wright, J. R. 2005. Immunoregulatory functions of surfactant proteins. *Nat. Rev. Immunol.* **5**: 58–68. [[Medline](#)] [[CrossRef](#)]