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Procollagen type III amino terminal propeptide concentrations in dogs with idiopathic pulmonary fibrosis compared with chronic bronchitis and eosinophilic bronchopneumopathy ☆

Henna P. Heikkilä [a,*], Emilie Krafft [b], Pascale Jespers [c], Kathleen McEntee [b,c], Minna M. Rajamäki [a], Cecile Clercx [b]

[a] Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, PO Box 57, 00014 University of Helsinki, Helsinki, Finland
[b] Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Liège, Bd de Colonster, Liège, Belgium
[c] Laboratory of Physiology, Faculty of Medicine, Université Libre de Bruxelles, Route de Lennik, Brussels, Belgium

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is characterised by an abnormal accumulation of collagen type III in the pulmonary interstitium. Procollagen type III amino terminal propeptide (PIIINP) is used as a marker of collagen type III synthesis. In this study, the concentrations of PIIINP were investigated in dogs with IPF ($n = 15$), dogs with chronic bronchitis (CB, $n = 19$), dogs with eosinophilic bronchopneumopathy (EBP, $n = 13$) and healthy dogs ($n = 25$). PIIINP concentrations in serum and bronchoalveolar lavage fluid (BALF) were analysed by radioimmunoassay. Serum PIIINP values did not differ between groups, indicating that serum PIIINP is not useful in evaluating respiratory diseases in dogs. BALF PIIINP was significantly elevated in dogs with IPF compared with healthy dogs ($P = 0.002$) and dogs with CB ($P < 0.001$). BALF PIIINP was significantly higher in dogs with EBP than in dogs with CB ($P = 0.003$) or healthy dogs ($P = 0.022$). There were no differences in BALF PIIINP concentrations between dogs with IPF and dogs with EBP or between dogs with CB and healthy dogs. These results indicate that IPF is associated with elevated BALF PIIINP concentrations. BALF PIIINP concentrations also are elevated in EBP, possibly due to secondary fibrotic changes.

Introduction

Canine idiopathic pulmonary fibrosis (IPF) is a chronic and progressive interstitial lung disease of unknown aetiology, poorly understood pathophysiology and a poor prognosis. IPF is recognised in humans (Liebow, 1975; Raghu et al., 2011), cats (Cohn et al., 2004; Williams et al., 2004) and dogs (Corcoran et al., 1999a; Lobetti et al., 2001; Norris et al., 2005; Heikkilä et al., 2011). In dogs, IPF predominantly is found in middle-aged and old West Highland white terriers (WHWTs), but other breeds can also be affected (Corcoran et al., 1999b; Lobetti et al., 2001).

IPF poses a diagnostic challenge for veterinary clinicians. The clinical picture of canine chronic bronchitis (CB) resembles that of IPF. Both diseases cause coughing and exercise intolerance (Kuehn, 2004; Heikkilä et al., 2011). Dogs with CB are of similar age and usually of small breeds (Kuehn, 2004). IPF and CB cannot be distinguished by bronchoscopy, since airway changes suggestive of concomitant bronchial disease can occur in dogs with IPF (Heikkilä et al., 2011). While the key histopathological feature of IPF is interstitial fibrosis (Norris et al., 2005), CB is characterised by long-standing airway inflammation and mucus hypersecretion (Wheeldon et al., 1977). Although CB is considered to be incurable, many dogs can be managed with anti-inflammatory treatment (Kuehn, 2004).

Unlike IPF and CB, canine eosinophilic bronchopneumopathy (EBP) usually affects young adult dogs. EBP is characterised by eosinophilic inflammation of the airways and pulmonary parenchyma. Most dogs cough, but the condition responds well to glucocorticoid therapy (Clercx and Peeters, 2007).

Differentiating IPF from other lung diseases, especially CB, can be difficult and may require high resolution computed tomography (HRCT) or histopathological examination of lung tissue. Thus, identification of a measurable marker of fibrosis could help to diagnose IPF in dogs (Krafft et al., 2011).

Procollagen type III amino terminal propeptide (PIIINP) is a marker of fibroblast activity and enhanced collagen type III turnover in humans. During synthesis of collagen type III, an amino terminal propeptide is cleaved from the procollagen molecule. This propeptide is then released into extracellular fluid and the circulation in proportion to the amount of collagen produced (Prockop et al.,

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* Corresponding author. Tel.: +358 503102664.
E-mail address: henna.heikkila@helsinki.fi (H.P. Heikkilä).

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1979). Abnormal accumulation of collagen types I and III in the lung interstitium occurs in IPF (Raghu et al., 1985; Norris et al., 2005) and elevated serum concentrations of PIIINP are present in humans with IPF (Lammi et al., 1999). Other respiratory diseases, such as chronic obstructive pulmonary disease (COPD) (Harju et al., 2010), sarcoidosis (Lammi et al., 1997), acute respiratory distress syndrome (Meduri et al., 1998) and infant bronchopulmonary dysplasia (Kaarteenaho-Wiik et al., 2004) also are associated with increased serum concentrations of PIIINP in humans.

A radioimmunoassay for measuring PIIINP has been validated in dogs and high PIIINP concentrations were present in a group of dogs with chronic bronchopneumopathy, mainly EBP (Schuller et al., 2006). The aim of the present study was to determine whether measurement of PIIINP in serum or bronchoalveolar lavage fluid (BALF) is useful in differentiating IPF from other chronic lung diseases in dogs.

Materials and methods

Patient selection and clinical examinations

Three groups of dogs with chronic respiratory disease (IPF, CB and EBP) and two groups of healthy control dogs (WHWTs and Beagles) were included. The IPF group contained 15 dogs (14 privately owned Finnish WHWTs and one Belgian Scottish terrier), 12 of which were included in our previous clinical study (Heikkilä et al., 2011). The diagnosis of IPF was confirmed by histopathology in 14/15 dogs after death. The diagnosis of the remaining dog was verified by HRCT. The diagnostic investigation included history and physical examination (15/15), haematology and serum biochemistry (13/15), faecal examination (10/15), arterial blood gas analysis (11/15), thoracic radiography (13/15), echocardiography (13/15), HRCT (7/15) and bronchoscopy with bronchoalveolar lavage (BAL) (13/15).

Serum and BALF samples were collected from 19 dogs of various breeds with CB examined at the Veterinary Teaching Hospitals of the University of Liége and the University of Helsinki (14 and 5 dogs, respectively). The diagnosis of CB was based on clinical signs and findings on thoracic radiography, bronchoscopy and BALF analysis (Kuehn, 2004). Other chronic respiratory and cardiac diseases were excluded. The diagnostic investigation also included haematology (19/19), serum biochemistry (5/19), faecal examination (8/19), arterial blood gas analysis (5/19), echocardiography (5/19) and HRCT (2/19).

Serum and BALF samples were collected from 13 dogs of various breeds diagnosed with EBP at the Veterinary Teaching Hospital of the University of Liége. The diagnosis was based on clinical signs, eosinophilia in BALF or bronchial eosinophilic infiltration and clinical response to glucocorticoids (Clercx and Peeters, 2007). Haematology (13/13), radiography (12/13), either faecal analysis (9/13) or therapeutic trial with fenbendazole (4/13) and histopathological examination of bronchial biopsies (12/13) were performed.

The control group comprised 13 healthy, privately owned Finnish WHWTs, which were recruited prospectively. All dogs had participated in our previous clinical study (Heikkilä et al., 2011). In addition, 12 healthy Beagles from the Faculty of Veterinary Medicine, University of Helsinki, were included. Healthy WHWTs underwent a similar diagnostic investigation to the dogs with IPF, except that blood gas analysis was performed in 11/13 dogs and HRCT, bronchoscopy and BAL were performed in 10/13 dogs. Haematology, serum biochemistry, thoracic radiography and bronchoscopy with BAL were performed in all Beagles. The study protocol was approved by the Committee for Experimental Animals of Western Finland (ESLH-2008-05403, approved 27 June 2008; HY 132-05, approved 19 October 2005).

Diagnostic procedures

Venous blood was drawn into plain tubes from the cephalic or jugular veins for collection of serum. BALF samples were collected by bronchoscopy under IV propofol-anaesthesia (PropoVet, Abbot Logistics; Diprivan, AstraZeneca). At least two different lung lobes were lavaged with sterile, warmed (37 °C) saline (either 60 mL divided into three aliquots or 2 mL/kg divided into two aliquots). The BALF sample was processed as described previously (Clercx et al., 2000; Rajamaki et al., 2001). BALF analysis consisted of total cell count (TCC) and differential cell count calculations and quantitative bacterial culture. Echocardiography and HRCT were performed as described elsewhere (Heikkilä et al., 2011). Faecal analysis included the Baermann method.

Procollagen type III amino terminal propeptide analysis

Serum and BALF PIIINP concentrations were measured in duplicate by radioimmunoassay (UniQ PIIINP radioimmunoassay, Orion Diagnostics). The method has been validated for use on canine serum and BALF (Schuller et al., 2006). Values below the detection limit (0.03 μg/L) were assigned an artificial value of 0.02 μg/L.

Statistical analysis

Normality of data distribution was evaluated by the Shapiro–Wilks test. Data are presented as mean and standard deviation (SD) or median and interquartile range (IQ). The groups were compared by analysis of variance (ANOVA) or Kruskal–Wallis one-way ANOVA on ranks. In the latter case, the Mann–Whitney rank sum test was used for pair-wise comparisons. Correlations were assessed with Spearman's correlation coefficient test. A receiver operating characteristic (ROC) curve was created to analyse the ability of BALF PIIINP to differentiate IPF from CB. All analyses were performed using PASW Statistics 18.0 for Windows. Differences were considered to be statistically significant at $P < 0.05$.

Results

Animals and clinical examinations

The sexes, breeds, ages and weights of animals are presented in Table 1. In the IPF group, the most common clinical signs were coughing and exercise intolerance (8/15 dogs). The mean duration of clinical signs was 14 months (range 2–29 months). Clinical, bronchoscopic, diagnostic imaging and histopathological findings of 11/15 dogs have been reported previously (Heikkilä et al., 2011).

All dogs with CB suffered from coughing, the mean duration of which was 11 months (range 2–60 months). All had changes on thoracic radiography, the most frequent being bronchointerstitial opacity, which was mainly moderate or severe (8 and 3 dogs, respectively). Alterations were evident in all dogs on bronchoscopy, including increased amounts of mucus (12/19), mucosal irregularity (9/19), congestion (9/19), bronchomalacia (6/19), mucosal thickening (3/19) and focal bronchiectasis (1/19). The median value for BALF TCC was 500 cells/μL (IQ 332–698 cells/μL, range 93–12,000 cells/μL). There was no significant bacterial growth from BALF and no parasites were detected on faecal examination.

Dogs with EBP all had coughing as their main clinical sign. In addition, four dogs were exercise-intolerant and two had dyspnoea. The mean age of onset of signs was 12 months (range 1–26 months). On thoracic radiography, bronchial opacity was detected in all dogs (severe in eight and moderate in four) and interstitial densities were visible in seven dogs (moderate in four, severe in three). One of the dogs had focal alveolar densities. Blood eosinophilia (eosinophil count >1900 cells/μL) was evident in 5/13 dogs. The median blood eosinophil count was 604 cells/μL (range 193–2924 cells/μL, IQ 302–1602 cells/μL). Bronchoscopic changes were present in all dogs, including increased amounts of mucus (10/13), mucosal irregularity (8/13), congestion (4/13) and mucosal thickening (2/13). The median TCC in BALF was 1580 cells/μL (range 400–12,900 cells/μL, IQ 925–4175 cells/μL) and the percentage of eosinophils ranged from 20–84%. In one dog, the proportion of eosinophils in BALF was relatively low (8%), but infiltration by eosinophils was detected on bronchial biopsy. There was no significant bacterial growth from BALF and no parasites were detected on faecal examination.

The control dogs had no clinical signs and no abnormalities were detected on ancillary examinations. Results of the diagnostic procedures conducted on the healthy WHWTs have been reported previously by Heikkilä et al. (2011).

Serum procollagen type III amino terminal propeptide concentrations

Serum PIIINP concentrations were measured in 13 dogs with IPF, nine dogs with CB, seven dogs with EBP and 13 healthy controls (all WHWTs). There were no statistically significant differences in serum PIIINP concentrations between groups (Fig. 1a).

Bronchoalveolar fluid procollagen type III amino terminal propeptide concentrations

BALF PIIINP concentrations were measured in 13 dogs with IPF, 19 dogs with CB, 13 dogs with EBP and 22 healthy control animals