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Airway Remodeling in Feline Lungs

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1 **Original article**

4 **Airway Remodeling in Feline Lungs**

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23    **Abstract**

24           Airway remodeling encompass structural changes that occur as the result of chronic  
25 injury and lead to persistently altered airway structure and function. Although this process  
26 is known in several human respiratory conditions such as asthma and chronic obstructive  
27 pulmonary disease (COPD), airway remodeling is poorly characterized in the feline  
28 counterpart. In this study, we describe the spontaneous pulmonary changes in three cats  
29 paralleling the airway remodeling reported in humans. We observed airway smooth muscle  
30 cells (ASMCs) hyperplasia (peribronchial and interstitial), airway subepithelial and  
31 interstitial fibrosis, and vascular remodeling by increased number of vessels in the  
32 bronchial submucosa. The hyperplastic ASMCs co-expressed  $\alpha$ -SMA, vimentin and  
33 desmin suggesting that vimentin, which is not normally expressed by ASMCs, may play a  
34 role in airway thickening and remodeling. ASMCs had strong cytoplasmic expression of  
35 TGF $\beta$ -1, which is known to contribute to tissue remodeling in asthma and in various  
36 bronchial and interstitial lung diseases, suggesting its involvement in the pathogenesis of  
37 ASMCs hyperplasia. Our findings provide histological evidence of airway remodeling in  
38 cats. Further studies on larger caseloads are needed to support our conclusions on the  
39 value of this feline condition as an animal model for nonspecific airway remodeling in  
40 humans.

41

42    **Keywords:** airway remodeling; airway smooth muscle cells hyperplasia; asthma; feline  
43 asthma; TGF $\beta$ .

## 44    **Introduction**

45            Airway remodeling is the set of processes encompassing morphological changes in  
46 structural cells of airways affected by chronic diseases, such as asthma or chronic  
47 obstructive pulmonary disease (COPD) (Prakash et al., 2017).

48

49            In human medicine, asthma is defined as a chronic inflammatory disorder  
50 characterized by airway hyper-responsiveness and remodeling that leads to thickening of  
51 the airway walls and to a variable degree of obstruction (Mims, 2015; Papi et al., 2018).

52

53            Analogously, airway obstruction in COPD is due to structural changes in the small  
54 airways, although they differ from those of human asthma (Chung, 2005; Sköld, 2010)

55

56            Airway remodeling comprises numerous cellular and extracellular alterations  
57 including mucous metaplasia of bronchial epithelium, inflammation, basement membrane  
58 thickening, subepithelial fibrosis, submucosal angiogenesis, increased number of  
59 myofibroblasts and airway smooth muscle cells (ASMCs) hyperplasia and hypertrophy  
60 (Fehrenbach et al., 2017; Grigoraş et al., 2016; Harkness et al., 2014; Kim et al., 2007;  
61 Pain et al., 2014; Prakash et al., 2017).

62

63            Cats and horses are recognized to be spontaneous animal models of asthma-like  
64 conditions (Aun et al., 2018), and Norris Reinero et al. (2004) demonstrated that cats  
65 develop pathologic changes similar to human patients. However, airway remodeling  
66 occurring in feline asthma has not been fully characterized (Masseau et al., 2015).

67

68            Different epithelial and mesenchymal cell types, such as bronchial epithelial cells  
69 fibroblasts, myofibroblasts and ASMCs, play a role in the pathogenesis of asthma,

70 producing cytokines and mediators promoting airway remodeling (Davies, 2009; Fixman et  
71 al., 2007; Rosethorne and Charlton, 2018). TGF $\beta$  is a central factor in epithelial-  
72 mesenchymal interactions (Saito et al., 2018) that mediates numerous fibrogenic  
73 responses, resulting in modifications of the extracellular matrix (ECM) (Pardali et al., 2017;  
74 Rockey et al., 2015). Moreover, TGF $\beta$  is involved in the remodeling of asthmatic lung  
75 disease, as well in idiopathic interstitial lung diseases and in COPD (Fitch et al., 2011;  
76 Michaeloudes et al., 2017) by promoting most of the processes underlying the  
77 morphological changes observed (Halwani et al., 2011), including differentiation of  
78 fibroblasts to myofibroblasts (Michalik et al., 2009) and proliferation of ASMCs (Chen and  
79 Khalil, 2006; Xie et al., 2007).

80

81 In this study, we describe the spontaneous pulmonary changes in three cats  
82 paralleling the pathological features of airway remodeling in human patients; further, we  
83 investigated the immunophenotype of hyperplastic ASMCs and the expression of TGF $\beta$ -1  
84 and its receptors TGF $\beta$  RI and TGF $\beta$  RII.

85

86 **Materials and methods**

87 *Necropsy and histology*

88 Pulmonary samples were collected from 3 client-owned cats, referred to the  
89 Pathology Service of the Department of Veterinary Medical Sciences for necroscopic  
90 examination. Cats were two males (No. 1; No. 2) and one female (No. 3), of 12, 14, and 12  
91 years of age respectively. Two out of three cases (case No. 1 and No. 2) were regularly  
92 vaccinated and annually subjected to anti-parasite therapy. In case No. 2, the medical  
93 history consisted of sporadic coughing episodes occurring in the summer period. Case No.  
94 3 had an unknown medical history. In cases Nos. 2 and 3 the anamnesis at the time of the

95 necropsy consisted in sudden death without previous clinical manifestations, whereas in  
96 case No. 1 death was preceded by respiratory distress.

97

98 The cats underwent necropsy, gross examination and tissue sampling for routine  
99 histological examination. All the biological samples used for the research were collected  
100 and processed in agreement with informed consent signed by the owners. Two lung  
101 samples were collected from each basal lobe (four samples/cat). In addition to H&E,  
102 sections of the lungs of each cat underwent Masson's trichrome stain (Bio-Optica, Milan,  
103 Italy). Three feline pulmonary samples without morphological alterations were used as  
104 control.

105

106 Collagen deposition in the alveolar interstitium was quantified on Masson's  
107 trichrome stained sections by image analysis using the software ImageJ (version 1.52t).  
108 For image analysis, 5 photomicrographs (area of each photomicrograph equal to 1.49  
109 mm<sup>2</sup>) from randomly selected 200x fields were used. For each photomicrograph, the ratio  
110 of positively stained area to the total parenchymal area (empty spaces were excluded) was  
111 used to quantify interstitial fibrosis (Supplementary S1).

112

113 The extent of collagen deposition within the bronchial submucosa (subepithelial  
114 fibrosis) was assessed in the examined cases and compared to controls.

115

116 *Immunohistochemistry*

117 Three micrometer-thick sections of lung were dewaxed and rehydrated.  
118 Endogenous peroxidase was blocked by immersion in 3% H<sub>2</sub>O<sub>2</sub> in methanol for 30' at  
119 room temperature. The primary antibodies, dilutions, antigen retrieval methods and tissues  
120 used as positive controls are reported in Table 1. Antigen retrieval was followed by cooling

121 at room temperature for 20'. Blocking of non-specific antigenic sites was achieved by  
122 incubating the slides in a solution of 10% goat serum in PBS for 30' at room temperature  
123 and afterwards incubated overnight at 4°C with the primary antibodies.  
124

125 Binding sites were revealed by secondary biotinylated antibody and amplified using  
126 a commercial avidin-biotin-peroxidase kit (ABC Kit Elite, Vector, Burlingame, CA). The  
127 chromogen 3,3'-diaminobenzidine (0.05%) (Histo-Line Laboratories, Emergo, Europe) was  
128 used. Slides were counterstained with Harris hematoxylin and permanently mounted with  
129 DPX medium.  
130

131 Positive internal and external controls were examined, and negative control slides  
132 were processed in parallel by replacing the primary antibody with a non-reactive isotype-  
133 matched antibody.  
134

135 A qualitative assessment of the number of small vessels in the bronchial  
136 submucosa was performed by comparing the CD31 immunolabeling in the examined  
137 cases with the control cases.  
138

#### 139 *Immunofluorescence staining*

140 Three micrometer-thick sections of lung were dewaxed in xylene; the primary  
141 antibodies, dilutions, antigen retrieval methods and tissues used as positive controls are  
142 reported in Table 1. Antigen retrieval was followed by cooling at room temperature for 20'.  
143 Blocking of non-specific antigenic sites was achieved by incubating the slides in a solution  
144 of 3% bovine serum albumin, 3% fetal bovine serum and 0.25% Triton X-100 in PBS for 1h  
145 at room temperature and afterwards incubated overnight at 4°C with the primary  
146 antibodies. Detection of primary antibodies was visualized with Alexa Fluor 488 and 555



147 (Abcam, Cambridge, UK). Sections were counterstained and mounted with Anti-Fade  
148 Fluorescence Mounting Medium with DAPI (Abcam, Cambridge, UK).

149

150 Slides were examined using a Nikon Eclipse Ni microscope equipped with the  
151 appropriate filter cubes to distinguish the fluorochromes used. Images were recorded  
152 using a Nikon DS-Qi1Nc digital camera and NIS Elements software BR 4.20.01 (Nikon  
153 Instruments Europe BV, Amsterdam, Netherlands).

154

## 155 **Results**

### 156 *Gross findings*

157 In cases No. 2 and 3, bilateral multifocal and patchy grayish-white lesions  
158 consistent with chronic interstitial pulmonary disease, localized mainly in caudal lobes,  
159 were identified. A mild and bilateral myocardial ventricular hypertrophy was found in case  
160 No. 2. Further minor macroscopic findings were found in the liver and kidneys and  
161 included mild hepatic congestion and lobular pattern accentuation in case No. 3; and  
162 diffuse, chronic, moderate interstitial nephritis in case No.2. In case No. 1 no evident gross  
163 alterations were found.

164

### 165 *Histopathology*

166 On microscopic examination, in all three cases, the most evident pulmonary lesion  
167 was a multifocal to coalescing interstitial thickening, consisting of bundles of spindle cells  
168 and a variable amount of collagen. Cells had abundant eosinophilic cytoplasm and small,  
169 oval to cigar-shaped and central basophilic nuclei and were interpreted as hyperplastic  
170 ASMCs. All cases had small- to medium-sized multifocal foci of ASMCs hyperplasia in  
171 terminal bronchioles, involving the whole parenchyma (Figs. 1A, 1B). Additionally, in case  
172 No. 3, the coalescence of hyperplastic ASMCs led to the formation of a focally extensive

173 lesion entrapping scattered bronchioles (Fig. 1C). Associated with extensive ASMCs  
174 hyperplasia, alveolar walls were multifocally lined by hyperplastic type II pneumocytes.  
175 Moderate peribronchial ASMCs hyperplasia were detected in each case. Multifocally,  
176 peribronchial and intraluminal bronchial mild inflammatory infiltrate of lymphocytes, plasma  
177 cells and numerous eosinophils was evident (Fig. 1D). Diffusely, the tunica media of  
178 pulmonary arteries was markedly hyperplastic.

179

180 Compared with controls, the alveolar interstitium of all three cats was thickened by  
181 fibrosis, based on image analysis assessment of Masson's trichrome stained sections (Fig.  
182 2) and by ASMCs hyperplasia.

183

184 The extent of fibrosis was mild (case No. 1) to moderate (cases Nos. 2 and 3) and  
185 the distribution was multifocal (cases Nos. 1 and 2) and multifocal to coalescing (case No.  
186 3), predominantly centered on the airways in all cases. In case No. 3, interstitial fibrous  
187 tissue was associated with the local extensive lesion characterized by ASMC hyperplasia  
188 (Supplementary S2).

189

190 Bronchial submucosa had hyperplastic glands admixed with an increased number  
191 of small vessels, while Masson's trichrome stain revealed an increased amount of collagen  
192 bundles beneath epithelium (subepithelial fibrosis) (Fig. 3).

193

194 Based on the evaluated criteria, the histopathological findings of each case are  
195 summarized in Table 2.

196

197 In all cases, histological examination of the liver revealed a diffuse and moderate  
198 congestion, interpreted as a peri-mortem finding. In case No. 3, a multifocal and moderate

199 hepatic lipidosis was observed, corresponding to the gross finding of accentuated lobular  
200 pattern. Examination of the myocardium revealed a mild, multifocal, interstitial fibrosis in all  
201 cases. Furthermore, in case No. 1, the myocardium was multifocally replaced by  
202 proliferation of fibroblasts and new thin-walled, delicate capillaries (angiogenesis)  
203 immersed in a loose extracellular matrix (granulation tissue). Tubulointerstitial  
204 lymphoplasmacytic nephritis was confirmed in case No. 2.

205

#### 206 *Immunohistochemistry*

207 Strong cytoplasmic expression of  $\alpha$ -SMA and TGF $\beta$ -1 was detected in hyperplastic  
208 ASMCs in all cases (Fig. 4B). TGF $\beta$ -1 was also expressed, but discontinuous and less  
209 intense, in normal ASMCs and less consistently in arterial smooth muscle cells (Fig. 4A).  
210 Cytoplasmic expression of TGF $\beta$  RI and RII was detected in bronchial epithelial cells and  
211 bronchial glands, and rarely within the cytoplasm of hyperplastic type II pneumocytes and  
212 in scattered alveolar macrophages (Fig. 4C, 4D). CD31 immunolabeling was identified  
213 within the endothelial cells lining of small vessels, showing an increased vascular density  
214 in the bronchial submucosa compared with control cases (Fig. 5).

215

#### 216 *Immunofluorescence staining*

217 Immunofluorescence staining revealed the cytoplasmic co-expression of  $\alpha$ -SMA,  
218 vimentin, and desmin in hyperplastic smooth muscle cells. Submucosal ASMCs always co-  
219 expressed desmin and  $\alpha$ -SMA, while vimentin immunolabeling was not detected in normal  
220 ASMCs (Fig. 6). A mildly positive vimentin stain was present in a few vascular smooth  
221 muscle cells of the arteries.

222

#### 223 **Discussion**

224           This report describes the histological features suggestive of airway remodeling in  
225 the lungs of three cats which parallels the changes reported in human chronic airway  
226 diseases.

227

228           In the lungs of all three cases, interstitial bundles of hyperplastic ASMCs were  
229 detected, involving the whole parenchyma. Lesions were more severe in small non-  
230 cartilaginous airways (e.g. case No. 3), similarly to changes observed in horses with  
231 pasture asthma (Ferrari et al., 2018). In humans, asthma-associated ASMCs remodeling  
232 involves both large and small airways (Elliot et al., 2015). On the contrary, feline asthma is  
233 classified as a bronchial disease, with bronchiolar involvement considered secondary  
234 change extending from bronchi (Reinero et al., 2019).

235

236           In all the pulmonary samples, the Masson's trichrome stain allowed to assessment  
237 of the increased amount of collagen in bronchial submucosa. This finding corresponds to  
238 subepithelial fibrosis, one of the histological changes of airway remodeling, that is  
239 mediated by resident fibroblasts, myofibroblasts (Brewster et al., 1990) and bone marrow  
240 derived-precursors (Nihlberg et al., 2006).

241

242           Among the structural changes found in our cases, we identified an increased  
243 number of small vessels in the bronchial submucosa, demonstrated by CD31  
244 immunolabeling. Angiogenesis and microvascular changes are common features of  
245 chronic airway disease and are referred to as vascular remodeling changes (Alagappan et  
246 al., 2013; Harkness et al., 2014; Keglowich and Borger, 2015; Saito et al., 2018).  
247 Morphological changes typical of this process include an increased number of bronchial  
248 vessels as well as an increased size of pulmonary vessels due to hyperplasia of tunica  
249 intima and media. In the cases described in this report, both lesions were identified.

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Hyperplasia and hypertrophy of the arteries is a common histologic finding in cats, recognized over time as a possible effect of lungworm infestation such as *Aelurostrongylus abstrusus* (Hamilton, 1970; Vezzosi et al., 2020). Furthermore, smooth muscle hyperplasia in the pulmonary arteries can be observed in severe equine asthma (Ceriotti et al., 2020). It remains to be clarified whether the thickening of the arterial walls observed in our cases may be linked to a vascular remodeling mechanism associated with airway remodeling in feline asthma or represents a sequela of lungworm infestation.

Pulmonary artery remodeling is known to be associated with increased pulmonary vascular resistance, which can lead to cardiac fibrosis (Siamwala et al., 2020); this could explain the cardiac interstitial fibrosis detected in the heart of the cases examined.

In all the cases included in the study, immunofluorescent staining revealed simultaneous expression of  $\alpha$ -SMA, vimentin and desmin in the hyperplastic interstitial ASMCs, whereas vimentin was not normally expressed in ASMCs.

Actin, desmin and vimentin are involved in smooth muscle cells contraction as well as in cell migration, and vimentin expression has been associated with an increased cellular motility (Tang and Gerlach, 2017). Since smooth muscle cells migration and motility is putatively related to thickening of smooth muscle in the asthmatic airways (Cleary et al., 2014; Tang and Gerlach, 2017), the increased vimentin expression found in the foci of ASMC hyperplasia may indicate an increased cellular motility in airway remodeling.

275 In healthy lung ,TGF $\beta$ -1 expression is confined to the airway epithelium, alveolar  
276 macrophages and fibroblasts (Coker et al., 1996; Kelley et al., 1991; Magnan et al., 1994),  
277 while in the three cases here described cytoplasmic expression of TGF $\beta$ -1 was detected in  
278 hyperplastic smooth muscle cells, mainly ASMCs and less consistently in vascular smooth  
279 muscle cells.

280

281 ASMCs are known to be synthetically active, producing and/or expressing several  
282 cytokines, including TGF $\beta$ -1, secondary to extracellular stimuli as occurs in chronic asthma  
283 (Howarth et al., 2004; Tliba and Panettieri, 2009). In asthmatic patients, increased  
284 immunoreactivity of TGF $\beta$ -1 and TGF $\beta$ -1 localization in submucosal smooth muscle cells  
285 is reported (Vignola et al., 1997), although eosinophils and fibroblasts are the main source  
286 of this growth factors. More recently, Xie et al., (2007) identified and increased TGF $\beta$ -1  
287 mRNA and protein expression in ASMCs of human asthmatic patients compared with non-  
288 asthmatics patients. The high expression of TGF $\beta$ -1 in hyperplastic ASMCs (Xie et al.,  
289 2007) and in structural ASMCs in the cases here described compared to controls, supports  
290 its role in the morphologic changes associated to these feline cases.

291

292 TGF $\beta$ -1 is a pleiotropic factor involved in different biological processes such as  
293 immune response, wound healing, tissue repair, and proliferation of fibroblasts (Xiao et al.,  
294 2012). In asthma, TGF $\beta$ -1 is responsible for the differentiation of fibroblasts, epithelial cells  
295 and also ASMCs into cells with higher contractile phenotype, thus contributing to increased  
296 airway hyper-responsiveness (Gawaziuk et al., 2007). Ojiaku et al. (2018) demonstrated  
297 that TGF $\beta$ -1 directly modulates cell shortening and increases contractility of human  
298 ASMCs; additionally, TGF $\beta$ -1 directly induces proliferation of smooth muscle cells (Chen  
299 and Khalil, 2006), with greater action in severe asthma (Perry et al., 2014).

300

301       The TGF $\beta$  signaling pathways occurs when one ligand of TGF family (e.g., TGF $\beta$ -1)  
302 binds to TGF $\beta$  RII, an intramembranous serine/threonine kinase receptor which then  
303 phosphorylates and activates TGF $\beta$  RI. The binding to the receptor complexes may  
304 activates both canonical SMAD-mediated and non SMAD-mediated cascade. Thus, the  
305 cellular response to TGF $\beta$  is regulated by the availability of receptors on the cell surface,  
306 which can be modified under certain conditions (Budi et al., 2017). So, we tested the  
307 expression of TGF $\beta$  receptors (TGF $\beta$  RI and TGF $\beta$  RII), hypothesizing a possible  
308 autocrine mechanism leading to ASMCs hyperplasia. Unexpectedly, hyperplastic ASMCs  
309 did not express TGF $\beta$  RI and TGF $\beta$  RII, which were nevertheless expressed in the  
310 cytoplasm of bronchial and glandular epithelium, scattered alveolar macrophages and type  
311 II pneumocytes. We speculate that, as previously reported (Gressner, 2011), in  
312 hyperplastic ASMCs the expression of TGF $\beta$ -1 might exert its function by an intracrine  
313 signaling involving the interaction of TGF $\beta$  with an unknown binding site in the intracellular  
314 domain of the Alk5 receptor with consequent activation of the non SMAD-mediated  
315 signaling pathway.

316

317       Considering the interstitial increase of collagen bundles, idiopathic pulmonary  
318 fibrosis (IPF) should be included among the differential diagnoses. A spontaneous,  
319 idiopathic pulmonary fibrosis-like condition has been described in cats with or without  
320 obvious respiratory clinical signs (Cohn et al., 2004; Williams et al., 2004). This chronic  
321 respiratory condition in domestic cats shows morphologic features similar to interstitial  
322 pneumonia typical of human IPF (Cohn et al., 2004; Evola et al., 2014; Williams et al.,  
323 2004): interstitial fibrosis, fibroblasts/myofibroblasts proliferation and enlarged alveolar  
324 spaces lined by bronchiolar epithelium (honeycombing), affecting mainly the subpleural

325 parenchyma (Travis et al., 2002). Moreover, ASMCs hyperplasia is commonly seen in IPF  
326 (Kanematsu et al., 1994). At least in one case (No. 3), the morphological changes  
327 resembled those described as “probable unusual interstitial (UIP) pattern” by Le Boedec et  
328 al., (2014) following human criteria (Raghu et al., 2011).

329

## 330 **Conclusions**

331 In cats, airway remodeling associated with feline asthma and other bronchial and  
332 interstitial disease remains a poorly characterized process. Considering that the cat  
333 represents a potential spontaneous animal model of asthma or interstitial fibrosis, it may  
334 be useful to know and characterize the lesions and pathogenesis of airway remodeling in  
335 the lungs of cats. We report the histological findings of airway remodeling in cats, and  
336 insights on the role of TGF $\beta$ -1 in their pathogenesis. Nevertheless, future studies on larger  
337 caseloads are needed to confirm our conclusions and to support the value of airway  
338 remodeling in cats as an animal model for nonspecific airway remodeling in humans.

339

## 340 **Conflict of interest statement**

341 The authors declare no conflict of interest.

342

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346

## 347 **Appendix: Supplementary material**

348 Supplementary data associated with this article can be found, in the online version,  
349 at doi: ...

350

## 351 **References**

352 Alagappan, V.K.T., de Boer, W.I., Misra, V.K., Mooi, W.J., Sharma, H.S., 2013.  
353 Angiogenesis and Vascular Remodeling in Chronic Airway Diseases. Cell



Biochemistry and Biophysics 67, 219–234.

Aun, M.V., Bonamichi-Santos, R., Arantes-Costa, F.M., Kalil, J., Giavina-Bianchi, P., 2018. Animal models of asthma: Utility and limitations. *Journal of Asthma and Allergy* 10, 293–301.

Brewster, C.E., Howarth, P.H., Djukanovic, R., Wilson, J., Holgate, S.T., Roche, W.R., 1990. Myofibroblasts and subepithelial fibrosis in bronchial asthma. *American journal of respiratory cell and molecular biology* 3, 507–511.

Budi, E.H., Duan, D., Derynck, R., 2017. Transforming Growth Factor- $\beta$  Receptors and Smads: Regulatory Complexity and Functional Versatility. *Trends in cell biology* 27, 658–672.

Cerioti, S., Bullone, M., Leclerc, M., Ferrucci, F., Lavoie, J.P., 2020. Severe asthma is associated with a remodeling of the pulmonary arteries in horses. *PLoS ONE* 15, 1–22.

Chen, G., Khalil, N., 2006. TGF- $\beta$ 1 increases proliferation of airway smooth muscle cells by phosphorylation of map kinases. *Respiratory Research* 7, 1–10.

Chung, K.F., 2005. The Role of Airway Smooth Muscle in the Pathogenesis of Airway Wall Remodeling in Chronic Obstructive Pulmonary Disease. *Proceedings of the American Thoracic Society* 2, 347–354.

Cleary, R.A., Wang, R., Waqar, O., Singer, H.A., Tang, D.D., 2014. Role of c-Abl tyrosine kinase in smooth muscle cell migration. *American journal of physiology. Cell physiology* 306, C753–C761.

Cohn, L.A., Norris, C.R., Hawkins, E.C., Dye, J.A., Johnson, C.A., Williams, K.J., 2004. Identification and Characterization of an Idiopathic Pulmonary Fibrosis–Like Condition in Cats. *Journal of Veterinary Internal Medicine* 18, 632–641.

Coker, R.K., Laurent, G.J., Shahzeidi, S., Hernández-Rodríguez, N.A., Pantelidis, P., du Bois, R.M., Jeffery, P.K., McAnulty, R.J., 1996. Diverse cellular TGF-beta 1 and TGF-beta 3 gene expression in normal human and murine lung. *The European respiratory journal* 9, 2501–2507.

Davies, D.E., 2009. The role of the epithelium in airway remodeling in asthma. *Proceedings of the American Thoracic Society* 6, 678–682.

Elliot, J.G., Jones, R.L., Abramson, M.J., Green, F.H., Mauad, T., McKay, K.O., Bai, T.R., James, A.L., 2015. Distribution of airway smooth muscle remodelling in asthma: Relation to airway inflammation. *Respirology* 20, 66–72.

Evola, M.G., Edmondson, E.F., Reichle, J.K., Biller, D.S., Mitchell, C.W., Valdés-Martínez, A., 2014. Radiographic and histopathologic characteristics of pulmonary fibrosis in nine cats. *Veterinary Radiology and Ultrasound* 55, 133–140.

Fehrenbach, H., Wagner, C., Wegmann, M., 2017. Airway remodeling in asthma: what really matters. *Cell and Tissue Research* 367, 551–569.

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Ferrari, C.R., Cooley, J., Mujahid, N., Costa, L.R., Wills, R.W., Johnson, M.E., Swiderski, C.E., 2018. Horses With Pasture Asthma Have Airway Remodeling That Is Characteristic of Human Asthma. *Veterinary Pathology* 55, 144–158.

Fitch, P.M., Howie, S.E.M., Wallace, W.A.H., 2011. Oxidative damage and TGF- $\beta$  differentially induce lung epithelial cell sonic hedgehog and tenascin-C expression: implications for the regulation of lung remodelling in idiopathic interstitial lung disease. *International journal of experimental pathology* 92, 8–17.

Fixman, E.D., Stewart, A., Martin, J.G., 2007. Basic mechanisms of development of airway structural changes in asthma. *European Respiratory Journal* 29, 379–389.

Gawaziuk, J.P., Ma, X., Sheikh, F., Cheng, Z.-Q., Cattini, P.A., Stephens, N.L., 2007. Transforming growth factor-  $\beta$  as a differentiating factor for cultured smooth muscle cells. *European Respiratory Journal* 30, 643–652.

Gressner, O.A., 2011. Intracrine signaling mechanisms of activin A and TGF- $\beta$ . *Vitamins and hormones* 85, 59–77.

Grigoraş, A., Grigoraş, C.C., Giuşcă, S.E., Căruntu, I.D., Amălinei, C., 2016. Remodeling of basement membrane in patients with asthma. *Romanian Journal of Morphology and Embryology* 57, 115–119.

Halwani, R., Al-Muhsen, S., Al-Jahdali, H., Hamid, Q., 2011. Role of transforming growth factor- $\beta$  in airway remodeling in asthma. *American Journal of Respiratory Cell and Molecular Biology* 44, 127–133.

Hamilton, J.M., 1970. The Influence of Infestation by *Aelurostrongylus Abstrusus* on the Pulmonary Vasculature of the Cat. *British Veterinary Journal* 126, 202–209.

Harkness, L.M., Kanabar, V., Sharma, H.S., Westergren-Thorsson, G., Larsson-Callerfelt, A.K., 2014. Pulmonary vascular changes in asthma and COPD. *Pulmonary Pharmacology and Therapeutics* 29, 144–155.

Howarth, P.H., Knox, A.J., Amrani, Y., Tliba, O., Panettieri, R.A., Johnson, M., 2004. Synthetic responses in airway smooth muscle. *The Journal of allergy and clinical immunology* 114, S32–S50.

Kanematsu, T., Kitaichi, M., Nishimura, K., Nagai, S., Izumi, T., 1994. Clubbing of the Fingers and Smooth-Muscle Proliferation in Fibrotic Changes in the Lung in Patients With Idiopathic Pulmonary Fibrosis. *Chest* 105, 339–342.

Keglowich, L.F., Borger, P., 2015. The Three A's in Asthma – Airway Smooth Muscle, Airway Remodeling & Angiogenesis. *The Open Respiratory Medicine Journal* 9, 70–80.

Kelley, J., Kovacs, E.J., Nicholson, K., Fabisiak, J.P., 1991. Transforming growth factor-beta production by lung macrophages and fibroblasts. *Chest* 99, 85S–86S.

Kim, E.S., Kim, S.H., Kim, K.W., Park, J.W., Kim, Y.S., Sohn, M.H., Kim, K.-E., 2007.

Basement membrane thickening and clinical features of children with asthma. *Allergy* 62, 635–640.

Le Boedec, K., Roady, P.J., O'Brien, R.T., 2014. A case of atypical diffuse feline fibrotic lung disease. *Journal of Feline Medicine and Surgery* 16, 858–863.

Magnan, A., Frachon, I., Rain, B., Peuchmaur, M., Monti, G., Lenot, B., Fattal, M., Simonneau, G., Galanaud, P., Emilie, D., 1994. Transforming growth factor beta in normal human lung: preferential location in bronchial epithelial cells. *Thorax* 49, 789–792.

Masseau, I., Banuelos, A., Dodam, J., Cohn, L.A., Reinero, C., 2015. Comparison of lung attenuation and heterogeneity between cats with experimentally induced allergic asthma, naturally occurring asthma and normal cats. *Veterinary Radiology and Ultrasound* 56, 595–601.

Michaeloudes, C., Kuo, C.-H., Haji, G., Finch, D.K., Halayko, A.J., Kirkham, P., Chung, K.F., Adcock, I.M., 2017. Metabolic re-patterning in COPD airway smooth muscle cells. *European Respiratory Journal* 50, 1700202.

Michalik, M., Pierzchalska, M., Legutko, A., Ura, M., Ostaszewska, A., Soja, J., Sanak, M., 2009. Asthmatic bronchial fibroblasts demonstrate enhanced potential to differentiate into myofibroblasts in culture. *Medical Science Monitor* 15, 194–201.

Mims, J.W., 2015. Asthma: Definitions and pathophysiology. *International Forum of Allergy and Rhinology* 5, S2–S6.

Nihlberg, K., Larsen, K., Hultgårdh-Nilsson, A., Malmström, A., Björner, L., Westergren-Thorsson, G., 2006. Tissue fibrocytes in patients with mild asthma: A possible link to thickness of reticular basement membrane? *Respiratory Research* 7, 50.

Norris Reinero, C.R., Decile, K.C., Berghaus, R.D., Williams, K.J., Leutenegger, C.M., Walby, W.F., Schelegle, E.S., Hyde, D.M., Gershwin, L.J., 2004. An experimental model of allergic asthma in cats sensitized to house dust mite or Bermuda grass allergen. *International Archives of Allergy and Immunology* 135, 117–131.

Ojiaku, C.A., Cao, G., Zhu, W., Yoo, E.J., Shumyatcher, M., Himes, B.E., An, S.S., Panettieri, R.A., 2018. TGF- $\beta$ 1 evokes human airway smooth muscle cell shortening and hyperresponsiveness via Smad3. *American Journal of Respiratory Cell and Molecular Biology* 58, 575–584.

Pain, M., Bermudez, O., Lacoste, P., Royer, P.J., Botturi, K., Tissot, A., Brouard, S., Eickelberg, O., Magnan, A., 2014. Tissue remodelling in chronic bronchial diseases: From the epithelial to mesenchymal phenotype. *European Respiratory Review* 23, 118–130.

Papi, A., Brightling, C., Pedersen, S.E., Reddel, H.K., 2018. Asthma. *The Lancet* 391, 783–800.

Pardali, E., Sanchez-Duffhues, G., Gomez-Puerto, M.C., ten Dijke, P., 2017. TGF- $\beta$ -induced endothelial-mesenchymal transition in fibrotic diseases. *International Journal*

of Molecular Sciences 18, 2157.

- Perry, M.M., Baker, J.E., Gibeon, D.S., Adcock, I.M., Chung, K.F., 2014. Airway smooth muscle hyperproliferation is regulated by microRNA-221 in severe asthma. *American journal of respiratory cell and molecular biology* 50, 7–17.
- Prakash, Y.S., Halayko, A.J., Gosens, R., Panettieri, R.A., Camoretti-Mercado, B., Penn, R.B., Aiyar, R., Ammit, A., Berkman, N., Bond, R., et al., 2017. An official American thoracic society research statement: Current challenges facing research and therapeutic advances in airway remodeling. *American Journal of Respiratory and Critical Care Medicine* 195, e4–e19.
- Raghu, G., Collard, H.R., Egan, J.J., Martinez, F.J., Behr, J., Brown, K.K., Colby, T. V., Cordier, J.F., Flaherty, K.R., Lasky, J.A., et al., 2011. An Official ATS/ERS/JRS/ALAT Statement: Idiopathic pulmonary fibrosis: Evidence-based guidelines for diagnosis and management. *American Journal of Respiratory and Critical Care Medicine* 183, 788–824.
- Reinero, C.R., Masseau, I., Grobman, M., Vientos-Plotts, A., Williams, K., 2019. Perspectives in veterinary medicine: Description and classification of bronchiolar disorders in cats. *Journal of Veterinary Internal Medicine* 33, 1201–1221.
- Rockey, D.C., Bell, P.D., Hill, J.A., 2015. Fibrosis — A Common Pathway to Organ Injury and Failure. *New England Journal of Medicine* 372, 1138–1149.
- Rosethorne, E.M., Charlton, S.J., 2018. Airway remodeling disease: Primary human structural cells and phenotypic and pathway assays to identify targets with potential to prevent or reverse remodeling. *Journal of Experimental Pharmacology* 10, 75–85.
- Saito, A., Horie, M., Nagase, T., 2018. TGF- $\beta$  Signaling in Lung Health and Disease. *International Journal of Molecular Sciences* 19, 2460.
- Siamwala, J.H., Zhao, A., Barthel, H., Pagano, F.S., Gilbert, R.J., Rounds, S., 2020. Adaptive and innate immune mechanisms in cardiac fibrosis complicating pulmonary arterial hypertension. *Physiological reports* 8, e14532.
- Sköld, C.M., 2010. Remodeling in asthma and COPD - differences and similarities. *The Clinical Respiratory Journal* 4, 20-27.
- Tang, D.D., Gerlach, B.D., 2017. The roles and regulation of the actin cytoskeleton, intermediate filaments and microtubules in smooth muscle cell migration. *Respiratory research* 18, 54.
- Tliba, O., Panettieri, R.A., 2009. Noncontractile functions of airway smooth muscle cells in asthma. *Annual review of physiology* 71, 509–535.
- Travis, W.D., King, T.E., Bateman, E.D., Lynch, D.A., Capron, F., Center, D., Colby, T. V., Cordier, J.F., DuBois, R.M., Galvin, J., et al., 2002. American thoracic society/European respiratory society international multidisciplinary consensus classification of the idiopathic interstitial pneumonias. *American Journal of Respiratory and Critical Care Medicine* 165, 277–304.

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- Vezzosi, T., Perrucci, S., Parisi, F., Morelli, S., Maestrini, M., Mennuni, G., Traversa, D., Poli, A., 2020. Fatal Pulmonary Hypertension and Right-Sided Congestive Heart Failure in a Kitten Infected with *Aelurostrongylus abstrusus*. *Animals* 10, 2263.
- Vignola, A.M., Chanez, P., Chiappara, G., Merendino, A., Pace, E., Rizzo, A., la Rocca, A.M., Bellia, V., Bonsignore, G., Bousquet, J., 1997. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. *American journal of respiratory and critical care medicine* 156, 591–599.
- Williams, K., Malarkey, D., Cohn, L., Patrick, D., Dye, J., Toews, G., 2004. Identification of spontaneous feline idiopathic pulmonary fibrosis: Morphology and ultrastructural evidence for a type II pneumocyte defect. *Chest* 125, 2278–2288.
- Xiao, L., Du, Y., Shen, Y., He, Y., Zhao, H., Li, Z., 2012. TGF-beta 1 induced fibroblast proliferation is mediated by the FGF-2/ERK pathway. *Frontiers in bioscience (Landmark edition)* 17, 2667–2674.
- Xie, S., Sukkar, M.B., Issa, R., Khorasani, N.M., Chung, K.F., 2007. Mechanisms of induction of airway smooth muscle hyperplasia by transforming growth factor-beta. *American journal of physiology. Lung cellular and molecular physiology* 293, L245-253.

581 **Tables**

582 **Table 1.** Immunohistochemistry and immunofluorescence materials and methods

583 information. INT, internal; EXT, external; CTR, control; MW, microwave; ON, overnight.

Marker	Type, Clone	Supplier	Dilution/ incubation	Ag retrieval	Positive INT and EXT CTR
<b>Immunohistochemistry</b>					
TGFβ-1	Mouse monoclonal anti TGFβ -1 (3C11)	Santa Cruz Biotechnology, California, USA	1:100/ON 4°C	10' Citrate pH6 MW:750W	Smooth muscle cells (INT)
CD31	Mouse monoclonal anti-PECAM- 1 (JC70)	Santa Cruz Biotechnology, California, USA	1:30/ON 4°C	10' EDTA pH8 MW:750W followed by 30' in Pepsin 0.05% at 37°	Endothelial cells (INT)
α-SMA	Mouse monoclonal anti- α-SMA (1A4)	Santa Cruz Biotechnology, California, USA	1:500/ON 4°C	10' Citrate pH6 MW:750W	Smooth muscle cells (INT)
TGFβ RI	Rabbit polyclonal IgG (T-19)	Santa Cruz Biotechnology, California, USA	1:200/ON 4°C	10' Citrate pH6 MW:750W	Granulation tissue (cat, skin) (EXT)
TGFβ RII	Rabbit polyclonal IgG (C-16)	Santa Cruz Biotechnology, California, USA	1:600/ON 4°C	10' Citrate pH6 MW:750W	Granulation tissue (cat, skin) (EXT)
<b>Immunofluorescence</b>					
Desmin	Rabbit polyclonal anti-Desmin (H76)	Santa Cruz Biotechnology, California, USA	1:20/ON 4°C	20' EDTA buffer pH9 pressure cooker (110- 120°C, high pressure)	Smooth muscle cells (INT)
Vimentin	Mouse monoclonal anti-Vimentin (V9)	Dako, Glostrup, Denmark	1:100/ON 4°C	20' EDTA buffer pH9 pressure cooker (110- 120°C, high pressure)	Mesenchymal cells (INT)
α-SMA	Mouse monoclonal anti- α-SMA (1A4)	Santa Cruz Biotechnology, California, USA	1:100/ON 4°C	20' EDTA buffer pH9 pressure cooker (110-	Smooth muscle cells (INT)

				120°C, high pressure)	
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585 **Table 2.** Pathological findings of each case according to histological criteria evaluated.

	<b>Case No. 1</b>	<b>Case No. 2</b>	<b>Case No. 3</b>
<b>ASMCs hyperplasia</b>	small- to medium foci in the peribronchial interstitium	small- to medium foci in the peribronchial interstitium	medium sized coalescent foci entrapping scattered bronchioles
<b>Subepithelial fibrosis</b>	Moderate, beneath bronchial epithelium	Moderate, beneath bronchial epithelium	Moderate, beneath bronchial epithelium
<b>Interstitial fibrosis</b>	Mild, multifocal	Moderate, multifocal	Moderate, multifocal to coalescent
<b>Airway inflammation</b>	peribronchial and mural mild inflammatory infiltrate*	peribronchial and mural mild inflammatory infiltrate*	peribronchial and mural mild inflammatory infiltrate*
<b>Microvascular changes</b>	Mild increased number of small vessels	Mild increased number of small vessels	Mild increased number of small vessels

586 \*The inflammatory infiltrate was characterized by lymphocytes, plasma cells and numerous eosinophils.

587

588 **Figures legend**

589 Fig. 1 Multifocal interstitial bundles of hyperplastic smooth muscle cells spread within  
590 pulmonary parenchyma in case No. 1 (A) and in case No. 2 (B); (C) Coalescent  
591 bundles of hyperplastic smooth muscle cells in case No. 3. H-E stain, Magnification  
592 100x. (D) Mild airways inflammation characterized by bronchial infiltrations of  
593 eosinophils; H-E, Magnification 200x.

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595 Fig. 2 Lung alveolo-septal interstitial collagen deposition in case No. 3 (B) compared with  
596 control case (A); Magnification 400x, Masson's trichrome stain. (C) quantitation by  
597 Image analysis show a higher content (fibrosis) in the 3 lungs compared to controls.

598  
599 Fig. 3 (A) Normal lower airway in control case (Magnification 40x); insert: detail  
600 (Magnification 400x). (B) diffuse subepithelial fibrosis in lower airways of case No. 2  
601 (Magnification 40x); insert: detail of increase in collagen bundles (Magnification 400x).  
602 Masson's trichrome stain.

603  
604 Fig. 4 (A) Immunohistochemical TGF $\beta$ -1 expression by airway smooth muscle cells and  
605 vascular smooth muscle cells of arteries detected in control cases; Magnification 40x.  
606 (B) Cytoplasmic immunohistochemical TGF $\beta$ -1 expression by hyperplastic smooth  
607 muscle cells in case No. 3; Magnification 40x. (C) Multifocal TGF $\beta$  RI cytoplasmatic  
608 expression by scattered pneumocyte type II and alveolar macrophages in case No. 2;  
609 Magnification 400x. (D) TGF $\beta$  RII cytoplasmatic expression by pneumocyte type II,  
610 alveolar macrophages and bronchial epithelial cells in case No. 2; Magnification 400x.

611  
612 Fig. 5 Immunohistochemical CD31 expression by endothelial cells of proliferated  
613 submucosal vessels (arrowheads) in case No. 2 (B, D) compared with control cases  
614 (A, C); Magnification 100x (A, B) and 400x (C, D).

615  
616 Fig. 6 Co-expression of desmin, alpha-smooth muscle actin ( $\alpha$ -SMA), and vimentin.  
617 Double immunofluorescence of desmin and  $\alpha$ -SMA (A, B) or vimentin (C, D) indicates  
618 co-expression of all three mesenchymal markers in the bundles of hyperplastic cells  
619 (B, D), suggesting an increased in motile capacity. However, normal ASMCs (internal  
620 control, C) did not express vimentin. Case No. 3, magnification 100x (A, C) and 400x  
621 (B, D).