

Article Type: Original

Epithelial basement membrane thickening is related to TGF-Beta 1 expression in children with chronic respiratory diseases

Lenka Hoňková¹, Jiří Uhlík², Katarína Beránková¹, Tamara Svobodová¹, Petr Pohunek¹

¹ Department of Pediatrics, ²nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic.

² Department of Histology and Embryology, ²nd Faculty of Medicine, Charles University in Prague, Prague, Czech Republic.

Running title: Bronchial remodelling in children.

Corresponding author: Prof Petr Pohunek, MD, PhD, Department of Pediatrics,

Motol University Hospital, V Úvalu 84, 150 06, Prague 5, Czech Republic

E-mail: petr.pohunek@LFMotel.cuni.cz, Tel: +420224432058, Fax: +420224432020

Key words: asthma, cystic fibrosis, primary ciliary dyskinesia, remodelling, TGF- β 1

Honkova L, Uhlík J, Berankova K, Svobodova T, Pohunek P.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.12275

This article is protected by copyright. All rights reserved.

Accepted Article

Epithelial basement membrane thickening is related to TGF-Beta 1 expression in children with chronic respiratory diseases

Pediatr Allergy Immunol

Abstract

Background: The complex structural changes of bronchial mucosa, known as remodelling, have been considered unique and typical for asthma. However, similar changes were recently found in other chronic respiratory diseases. The aim of this study was to compare basement membrane (BM) thickness and the number of transforming growth factor beta 1 (TGF- β 1) positive epithelial cells in children with asthma, cystic fibrosis (CF), primary ciliary dyskinesia (PCD) and healthy controls.

Methods: A total of 58 children (11.1 $\pm$ 3.9 yrs, 55% males) were enrolled in this cross-sectional study. Endobronchial biopsy was performed in 27 children with asthma, 12 with CF, 12 with PCD and in 7 control patients. We studied the samples using light microscopy to assess BM width, the number of TGF- β 1 positive epithelial cells and their correlation.

Results: We found increased BM thickness (6.65 $\pm$ 1.22 μ m vs. 2.93 $\pm$ 0.75 μ m, p <0.01) and a higher number of TGF- β 1 positive epithelial cells (61.39 $\pm$ 19.03 vs. 21.57 $\pm$ 12.58, p <0.01) in children with chronic respiratory diseases compared to controls. There was no difference in these parameters between asthma, CF and PCD. A positive correlation between BM thickness and the number of TGF- β 1 positive cells was observed in all groups including controls (r =0.84, p <0.01).

Conclusions: Increased BM thickness and number of TGF- β 1 positive epithelial cells were found in children with asthma, CF and PCD. The number of TGF- β 1 positive cells correlated positively with the BM thickness in all groups. We suggest that this might be a common generic feature of bronchial remodelling in chronic respiratory diseases.

Key words: asthma, cystic fibrosis, primary ciliary dyskinesia, remodelling, TGF- β 1

Corresponding author: Prof Petr Pohunek, MD, PhD, Department of Pediatrics, Motol University Hospital, V Úvalu 84, 150 06, Prague 5, Czech Republic

Introduction

Chronic respiratory diseases in childhood comprise a significant part of pediatric clinical practice. Bronchial asthma is the most prevalent chronic respiratory disease in children. The worldwide prevalence of the disease has been rising in recent years (1). Cystic fibrosis (CF) is the most common genetic disease in Caucasian populations with an autosomal recessive pattern of inheritance. Primary ciliary dyskinesia (PCD) is a much less prevalent autosomal recessive genetic disorder. These three diagnoses share several common characteristics. First, their symptoms usually appear in early childhood and are followed by variable course of the disease which is modified by treatment. Second, they can all present with respiratory symptoms ranging from intermittent cough to serious dyspnoea due to severe bronchial obstruction (2,3). These symptoms may be misleading in the search for a correct diagnosis.

The complex of structural changes known as airway remodelling has been considered a unique and typical feature of asthmatic airways (4). These changes include increased epithelial fragility, basement membrane (BM) thickening, subepithelial fibrosis, deposition of extracellular matrix proteins and proteoglycans, increased smooth muscle mass, mucus gland hypertrophy and hyperplasia and new vessel formation (4-7). The resulting thickening of the bronchial wall has been considered the main reason for the development of airway hyperresponsiveness leading to airflow obstruction and a progressive lack of airway distensibility (8). Airway remodelling in children with asthma has been confirmed in children with active disease as early as in the second year of life (9). Recently, the first signs of airway remodelling were described in young children at risk of developing asthma even before the confirmation of diagnosis (10,11).

Transforming growth factor beta 1 (TGF- β 1) is produced by various inflammatory and structural cells and stimulates fibroblast proliferation, affects the synthesis of extracellular matrix proteins, down-regulates proteolytic enzymes and increases the expression of receptors for extracellular matrix proteins (12-15). It has been found to play an important role in airway wall thickening in patients with asthma (16). Furthermore, in asthma it was shown that TGF- β 1 may initiate the process described as epithelial-mesenchymal transition. It is speculated that these TGF- β 1 activated epithelial cells may play a prominent role in the deposition of extracellular proteins that leads to increased BM thickness (17). On the other hand, there are currently very limited data on the role of TGF- β 1 in other chronic respiratory diseases (18).

The aim of this study was to compare BM thickness and the number of TGF- β 1 positive epithelial cells in children with asthma, CF, PCD and healthy controls in order to clarify the pattern of bronchial wall remodelling in various chronic respiratory airway disorders.

Methods

Patients

A total of 58 patients aged 4 to 19 years (11.1 ± 3.9 yrs, 55% males) were enrolled in this cross-sectional study. All patients underwent flexible bronchoscopy for clinical reasons at the Pediatric Department of Motol University Hospital in Prague between October 2010 and March 2013. Besides samples required for the clinical diagnostic assessment, an additional endobronchial biopsy was obtained for the purpose of this study. Twenty-seven children were diagnosed with moderate-to-severe asthma, twelve patients with CF and twelve patients with PCD. The diagnoses of asthma, CF and PCD were made according to standard criteria (19-21). The control group consisted of seven patients. Five patients were investigated for acute or recurrent respiratory symptoms and no lower respiratory airway disease was found. In two cases, a bronchial biopsy was performed in children with no respiratory morbidity that required bronchoscopy-assisted intubation for a gastroscopy. Patients in the control group were all non-atopic (including clinical presentation, family and personal histories) with normal results of pulmonary function testing. Anonymity of the research specimens was guaranteed according to the standards of our institution. The study was approved by the local ethics committee. Legal representatives of all the study subjects gave written informed consent for the research biopsy and participation in the study.

Clinical testing

In each patient, a full medical history was obtained; special attention was paid to the history of oral or inhaled corticosteroids (ICS). Basic allergic markers were tested (serum total IgE, serum specific IgE and skin prick tests). The presence of atopy was defined as a positive skin reaction (wheal diameter >3 mm above the negative control response) to the mix of allergens Alyostal® (Stallergenes S.A., Paris, France) and/or an increase in total or specific serum IgE tested by AlaTOP® (Siemens AG, Erlangen, Germany). Before bronchoscopy, lung function testing was performed using a flow-volume loop on an open circuit spirometer Zan 300 (Zan Messgeräte GmbH, Oberthulda, Germany). A positive bronchodilator response was defined as a forced expiratory volume in 1s (FEV_1) improvement of more than 12% of the baseline measurement after the inhalation of 4 doses of 100 µg of salbutamol administered by a metered dose inhaler via a Volumatic® spacer (GlaxoSmithKline, Brentford, United Kingdom).

Flexible bronchoscopy

Flexible bronchoscopy was performed using a standard protocol under general anaesthesia using a combination of intravenous propofol and inhaled sevoflurane with preserved spontaneous ventilation. The size of the bronchoscope was determined according to the age of the child. A videobronchoscope Olympus BF 3C160 was used for children aged up to 5 or 6 years (3.6 mm distal tip outer diameter with a 1.2 mm working channel) and a videobronchoscope Olympus BF P180 for older children (4.9 mm distal tip outer diameter with a 2.0 mm working channel). After visual bronchoscopic evaluation and collection of all clinically relevant specimens, another endobronchial biopsy was performed for the purpose of the study. A biopsy forceps Olympus FB-21C-1 or FB-56D-1 according to the appropriate bronchoscope diameter was used. (All bronchoscopic instruments by Olympus Europe GmbH, Hamburg, Germany). The biopsies were taken from the carinas of the segmental or subsegmental bronchi of the right lower lobe. All children tolerated the bronchoscopy well and the collection of research biopsies was thus possible in all subjects.

Bronchial biopsies

Immediately after the biopsy, the specimen was fixated for 4 hours in a 4% solution of fresh paraformaldehyde dissolved in phosphate buffered saline (PBS) adjusted to pH 7.4. Subsequently, the samples were transferred to the same PBS and maintained at a temperature of 4°C overnight. Then, the specimens were dehydrated by processing through alcohol in a series of increasing concentrations, cleared by chloroform and embedded in paraffin. The specimens were cut into 5 µm thick sections.

Haematoxylin-eosin stained samples were used to analyse the BM thickness using the light microscope Axioskop (Zeiss, Oberkochen, Germany) or BX53 (Olympus, Tokyo, Japan) equipped with a CCD camera ProgRes C5 (Jenoptik, Jena, Germany) and computer image analysis software NIS Elements AR (Laboratory Imaging, Prague, Czech Republic). The BM thickness was assessed by taking measurements at 50 µm intervals along the BM length. The required minimum BM length in every sample was at least 1000 µm. All measurements were made by the same investigator (LH) who was blinded to the clinical data.

The method of indirect immunohistochemistry was used for the analysis of the TGF-β1 positive epithelial cells using rabbit polyclonal antibody to TGF-β1 Ab66043 (Abcam, Cambridge, United Kingdom) at a dilution of 1:200 with PBS. The specimens were processed by the Expose rabbit-specific HRP/DAB detection IHC kit Ab80437 (Abcam, Cambridge, United Kingdom) according

to the manufacturer's instructions. Briefly, after blocking of endogenous enzyme activity, the antigen retrieval was performed by heating the specimen at the temperature of 92°C for 5 minutes in citrate buffer (pH 6). Subsequently, the Protein Block was applied to block non-specific background staining. The overnight incubation with primary antibody followed the incubation with HRP Conjugate. After short visualisation of the specimens with a mixture of DAB Chromogen and DAB Substrate, the nuclei were briefly counterstained with haematoxylin. The number of TGF- β 1 positive epithelial cells was counted as a number of positive cells per unit length (1 mm) of BM. Only cells containing nucleus in the section were included in the evaluation. The required minimum BM length was 1000 μ m.

Statistical analysis

Normally distributed data are presented as means $\pm$ standard deviation ($\pm$ SD) and non-normally distributed data as medians with interquartile range (IQR). The distribution of data was evaluated by the Kolmogorov-Smirnov test. Fisher's test, Mann-Whitney U-test, Kruskal-Wallis and Spearman rank correlation were used when appropriate. P value <0.05 was considered to indicate a statistically significant difference.

Results

Baseline characteristics of all patients are shown in Table 1. Bronchial biopsy specimens were taken from all 58 subjects using flexible bronchoscopy. At least 1 representative histological sample was available for analysis in all subjects.

All data of the BM measurement and the TGF- β 1 positive epithelial cell counts are summarised in Table 1. Increased BM thickness was found in children with chronic respiratory diseases (asthma, CF and PCD combined) compared to healthy controls ($p<0.01$) (Fig. 1,2). The number of TGF- β 1 positive epithelial cells was also higher in children with chronic respiratory diseases compared to controls ($p<0.01$) (Fig. 3,4). Increased BM thickness and a higher number of TGF- β 1 positive epithelial cells were found in each group (asthma, CF, PCD) compared to healthy controls, but no differences were found between these groups. The number of TGF- β 1 positive epithelial cells positively correlated with the thickness of BM in all observed groups: asthma ($r=0.82$, $p<0.01$); CF ($r=0.88$, $p<0.01$); PCD ($r=0.90$, $p<0.01$); asthma, CF and PCD combined ($r=0.84$, $p<0.01$); and in controls ($r=0.82$, $p<0.01$) (Fig. 5). Positive staining for TGF- β 1 was observed in the perinuclear and apical parts of ciliated and basal bronchial epithelial cells.

No correlation between the BM thickness or number of TGF- β 1 positive cells and FEV₁ was found ($r=-0.027$, $p=0.85$; $r=0.078$, $p=0.59$). There was no difference between atopics and non-atopics ($p=0.75$), also no correlation between age and BM thickness was found ($r=0.001$, $p=0.79$).

Discussion

In this study we have confirmed our hypothesis that structural changes that are typical for remodelling of asthmatic airways can also be found in other chronic respiratory diseases presenting with bronchial obstruction in childhood. In addition, to the best of our knowledge, this is the first study also addressing the relationship of TGF- β 1 positive epithelial cells and BM thickening in children with various chronic respiratory diseases.

Thickening of the reticular layer of the BM in patients with asthma is a generally accepted sign of airway remodelling and was described in patients suffering from asthma even in an early age (10,11,22). There is much less information available about remodelling processes in other airway diseases. Moreover, the few published studies have shown conflicting results. The study by Hubeau et al. found BM thickening in adult CF patients to be a possible consequence of a chronic inflammatory process (23). In contrast, Durieu et al. found the BM to be significantly thinner when compared to healthy controls and suggested that this might be the result of proteolytic degradation (24). In our study, we have found significant BM thickening in asthma, CF and PCD patients compared to healthy controls.

The numbers of TGF- β 1 positive cells positively correlated with the BM thickness in the studied samples. This finding is in agreement with previous *in vitro* and *in vivo* studies which confirmed the role of TGF- β 1 in proliferative and reparation processes in adults with chronic bronchitis and asthma (25,26). Additionally, Nakamura et al. demonstrated the importance of TGF- β 1 in BM formation in an *in vitro* study on human kidneys (27).

Positive correlation of BM thickening and TGF- β 1 concentration in bronchoalveolar lavage fluid in children was previously found in the study of Hilliard et al., which is to date the only study that has described BM thickening in children with CF and PCD (18). Compared to this study, we have more accurately defined groups of patients that allowed the comparison of CF and PCD with the established changes in asthmatics. Moreover, our results indicate the more exact localisation of TGF- β 1 production within the tissue, noting that epithelial cells might be an important source of

increased TGF- β 1. Epithelial TGF- β 1 is thought to induce the activation of fibroblasts and myofibroblasts (28). The production of TGF- β 1 by activated epithelial cells might thus be an essential step in the initiation and further development of structural changes in the bronchial mucosa (26). Based on our results, we suggest that the influence of epithelial TGF- β 1 on deeper layers of the bronchial mucosa might be a universal pathogenetic mechanism causing the development of further remodelling, irrespective of the primary disease.

We have observed positive immunostaining for TGF- β 1 also in the epithelial cells of healthy controls. However, the number of TGF- β 1 positive cells was significantly lower compared to patients with chronic respiratory diseases. Interestingly, the number of TGF- β 1 positive cells also correlated with BM thickness in healthy children. This is in agreement with the results of the study by Magnan et al. who described the expression of TGF- β 1 in the epithelial cells of healthy human lungs (29). These findings illustrate the role of TGF- β 1 not just as a potent mediator of the inflammatory process but also as an essential cytokine necessary for common reparation processes in healthy lungs.

This study should be understood in the context of its limitations. Generally, inherent limitations of this sort of cross-sectional study exist. The accidental nature of taking samples according to clinical indications led to the children included in our study comprising a wide range of ages, disease activity and severity. Moreover, ethical aspects of invasive examinations in pediatric patients always significantly reduce the number of healthy controls enrolled. Nevertheless, this pilot study is one of the largest studies published to date on this topic.

The majority of observed children were treated with ICS at the time of bronchoscopy. Although there are some studies suggesting that corticosteroid treatment has no significant effect on TGF- β 1 expression in humans, this might be a potential confounding factor in this study (30). However, the proportion of patients using ICS was not significantly different between the observed groups.

In conclusion, in this study we have found increased BM thickness related to the number of TGF- β 1 positive epithelial cells in children with asthma, CF and PCD. This finding indicates the important role of bronchial epithelial cells and TGF- β 1 in the development of remodelling in chronic respiratory diseases. We suggest that these changes are not a unique feature of asthma, but might be closely related to common generic processes such as chronic epithelial irritation and inflammation. The reaction of the airway epithelium stimulated by inflammatory mediators seems to be stereotyped irrespective of the pathogenesis of the particular respiratory disease.

Funding

The study was supported by following research grants: IGA MZ NT/11444 (Ministry of Health, Czech Republic) and GAUK 360213 (Charles University in Prague, Czech Republic).

Acknowledgments

The authors are grateful to Eva Hansvenclova and Jakub Honek for their assistance with the statistical analysis, Tereza Jenik for the inclusion of her patients in the study and for performing bronchoscopy, Katrin Ctvrtnikova for her help with the preparation of biopsy material. They also gratefully acknowledge the patients and families who agreed to take part in the study.

References

- 1 Asher MI, Montefort S, Björkstén B et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multi-country cross-sectional surveys. *The Lancet* 2006; 368: 733-743.
- 2 Ormeford LP, Thomson RA, Anderson CM, et al. Reversible airway obstruction in cystic fibrosis. *Thorax* 1980; 35: 768-772.
- 3 Hellinckx J, Demedts M, De Boeck K. Primary ciliary dyskinesia: Evolution of pulmonary function. *Eur J Pediatr* 1998; 157: 422-426.
- 4 Jeffery PK. Remodelling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 2001; 164: S28-S38.
- 5 Baraldo S, Turato G, Bazzan E, et al. Noneosinophilic asthma in children: relation with airway remodelling. *Eur Respir J*. 2011; 38: 575-83.

6 Turato G, Barbato A, Baraldo S, et al. Nonatopic children with multitrigger wheezing have airway pathology comparable to atopic asthma. *Am J Respir Crit Care Med* 2008; 178: 476-82.

7 Payne DN, Rogers AV, Adelroth E, et al. Early thickening of the reticular basement membrane in children with difficult asthma. *Am J Respir Crit Care Med* 2003; 167:78-82.

8 Jeffery PK, Wardlaw AJ, Nelson FC, et al. Bronchial biopsies in asthma: an ultrastructural, quantitative study and correlation with hyperactivity. *Am J Respir Crit Care Med* 1989; 140: 1745-1753.

9 Saglani S, Payne ZD, Zhu J et al. Early detection of airway remodelling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med* 2007; 179: 858-864.

10 Pohunek P, Warner JO, Turzikova J et al. Markers of eosinophilic inflammation and tissue remodelling in children before clinically diagnosed bronchial asthma. *Pediatr Allergy Immunol* 2005; 16: 43-51.

11 Berankova K, Uhlik J, Honkova L, et al. Structural changes in the bronchial mucosa of young children at risk of developing asthma. *Pediatr Allergy Immunol* 2014; 25: 136-142.

12 Hill DJ, Strain AJ, Elstow SF, et al. Bifunctional action of transforming growth factor- β on DNA synthesis in early passage human foetal fibroblasts. *J Cell Physiol* 1986; 128: 322-328.

13 Edwards DR, Murphy G, Reynolds JJ, et al. Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. *EMBO J* 1987; 6: 1899-1904.

14 Igotz RA, Massague J. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J Biol Chem* 1986; 261: 4337-4345.

15 Howat WJ, Holgate ST, Lackie PM. TGF- β isoform release and activation during *in vitro* bronchial epithelial wound repair. Am J Physiol Lung Cell Mol Physiol 2002; 282: L115-L123.

16 Ohno I, Nitta Y, Yamauchi K, et al. Transforming growth factor β 1 (TGF β 1) gene expression by eosinophils in asthmatic airway inflammation. Am J Resp Cell Mol Biol 1996; 15: 404-409.

17 Hackett TL, Warner SM, Stefanowicz D et al. Induction of epithelial- mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factor β 1. Am J respire Crit Care Med 2009; 180: 122-133.

18 Hilliard TN, Regamey N, Shute JK, et al. Airway remodelling in children with cystic fibrosis. Thorax 2007; 62: 1074-1080.

19 American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am Rev Respir Dis 1987; 136:225-244.

20 Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. J Pediatr 1998; 132: 589-595.

21 Bush A, Cole P, Hariri M, et al. Primary ciliary dyskinesia: diagnosis and standards of care. Eur Respir J 1998; 12: 982-988.

22 Jeffery PK. Remodelling in asthma and chronic obstructive lung disease. Am J Respir Crit Care Med 2001; 164: S28-S38.

23 Hubeau C, Lorenzato M, Couetil JP et al. Quantitative analysis of inflammatory cells infiltrating the cystic fibrosis airway mucosa. Clin Exp Immunol 2001; 124: 69-76.

24 Durieu I, Peyrol S, Gindre D, et al. Subepithelial fibrosis and degradation of the bronchial extracellular matrix in cystic fibrosis. Am J Respir Crit Care Med 1998; 158: 580-588.

25 Nakamura Y, Tate L, Ertl RF, et al. Bronchial epithelial cells regulate fibroblast proliferation. Am J Physiol 1995; 269: L377-L387.

26 Vignola AM, Chanez P, Chiappara G, et al. Transforming growth factor- β expression in mucosal biopsies in asthma and chronic bronchitis. Am J of Resp Crit Med 1997; 156: 591-599.

27 Nakamura T, Miller D, Ruoslahti E, et al. Production of extracellular matrix by glomerular epithelial cells is regulated by transforming growth factor-beta 1. Kidney Int. 1992; 41: 1213-1221.

28 Holgate ST, Davies DE, Lackie PM et al. Epithelial-mesenchymal interactions in the pathogenesis of asthma. J Allergy Clin Immunol 2000; 105: 193-204.

29 Magnan A, Frachon I, Rain B, et al. Transforming growth factor beta in normal human lung: preferential location in bronchial epithelial cells. Thorax 1994; 49: 789-792.

30 Chakir J, Shannon J, Molet S, at al. Airway remodelling associated mediators in moderate to severe asthma: effects of steroids on TGF-beta, IL-11,IL-17, and type I and III collagen expression. J Allergy Clin Immunol 2003; 111: 1293-1298.

FIGURE TITLES AND LEGENDS

Figure 1. Basement membrane (arrows) in the bronchial mucosa: (a) asthmatic patient; (b) patient with cystic fibrosis; (c) patient with primary ciliary dyskinesia; (d) control subject; haematoxylin-eosin, original magnification x 40.

Figure 2. Basement membrane (BM) thickness in patient groups. BM thickness was significantly greater in all of the studied groups compared to controls; CF, cystic fibrosis; PCD, primary ciliary dyskinesia.

Figure 3. Transforming growth factor beta 1 (TGF- β 1) immunoreactivity of the bronchial mucosa. Examples of TGF- β 1 positive epithelial cells are labeled by arrows: (a) asthmatic patient; (b) patient with cystic fibrosis; (c) patient with primary ciliary dyskinesia; (d) control subject; original magnification x 40.

Figure 4. The number of transforming growth factor beta 1 (TGF- β 1) positive epithelial cells per 1 mm of the basement membrane length in patient groups. TGF- β 1 epithelial positivity was significantly greater in all studied groups compared to healthy controls; CF, cystic fibrosis; PCD, primary ciliary dyskinesia.

Figure 5. Correlation between the transforming growth factor beta 1 positive epithelial cells per 1 mm of the basement membrane (BM) length and the BM thickness. Spearman rank correlation shown with 95% confidence intervals; CF, cystic fibrosis; PCD, primary ciliary dyskinesia.

Table 1 Baseline characteristics, basement membrane thickness and number of TGF- β 1 positive epithelial cells in each patient group

	Asthma (n=27)	CF (n=12)	PCD (n=12)	Asthma+CF+PCD (n= 51)	Controls (n=7)
Age (years)	9.9 (4.4-16.7)	11.1 (5.8-16.2)	13.1 (4.5-19.0)	11.0 (4.4-19.0)	12.0 (5.8-17.2)
Sex (males %)	78	33	33	57	43
ICS (%)	85*	67*	67*	76*	0
OCS (%)	0	0	0	0	0
FEV ₁ (% predicted)	95 (85-102)	76 (49-97)	84 (75-94)	92 (75-99)	89 (84-113)
Atopy (%)	56*	58*	17	47*	0
BM thickness (μ m)	6.4	6.2	6.5	6.4	2.6

	(5.7-7.8) *	(5.7-6.9) *	(6.2-7.9) *	(5.8-7.7) *	(2.4-3.5)
TGF- β 1 positive epithelial cells (1/mm)	59	54	59	57	18
	(48-75) *	(43-63) *	(49-86) *	(48-72) *	(9-30)

BM, basement membrane; CF, cystic fibrosis; FEV₁, forced expiratory volume in 1s; ICS, inhaled corticosteroids; OCS, oral corticosteroids; PCD, primary ciliary dyskinesia; TGF- β 1, transforming growth factor beta 1

*p<0.05 for the respective group vs. controls

Values of age are median (range), other values are median (interquartile range).



