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Relationship between airway basement membrane thickness and lung function tests in patients with asthma

Abstract

Introduction: Airway remodeling is a characteristic feature of asthma. It is believed that airway remodeling affects lung function and bronchial hyper-responsiveness. Therefore, the relationship between remodeling and lung function is still a matter of extensive research. However, the results of many studies are inconsistent.

The aim of the study was to assess the relationship between lung function parameters and basement membrane (BM) thickness in patients with asthma.

Material and methods: Twenty asthma patients were chosen for the study (ten male, ten female, mean age 37 $\pm$ 15 yrs). Ten were newly diagnosed, steroid-naive patients and the other ten were patients known to have asthma who had not been treated with steroids for at least three months. The study group was selected based on the results of: clinical assessment, allergic skin-prick tests, lung function testing and bronchial challenge with methacholine. Nine (45%) patients had chronic mild, nine (45%) had moderate and two (10%) had intermittent asthma. Mean FEV1% pred. was 83 $\pm$ 18, mean FEV1%VC 69 $\pm$ 9, mean FVC% pred. 101 $\pm$ 14. All patients underwent research fiberoptic bronchoscopy with BAL and bronchial mucosal biopsies. Light-microscopic measurements of BM thickness were performed in hematoxylin-eosin stained slides of bronchial wall specimens with semi-automatic software analysis MultiScan Base 08.98.

Results: Mean BM thickness was 12.8 $\pm$ 2.8 μm (range: 8.5–20.7 μm). No significant correlations between BM thickness and FEV1% pred., FEV1%VC, FVC% pred., RV% pred., TLC% pred., Raw (pre- and post-bronchodilator) and PC_{20} were observed.

Conclusions: In our group of asthma patients, mean BM was significantly thickened. No relationship between BM thickness and lung function tests, including hyper-responsiveness, was found.

Key words: basement membrane, airway remodeling, lung function tests, hyper-responsiveness, asthma

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Introduction

Airway basement membrane (BM) thickening in patients who died during asthma exacerbation was observed as long ago as the early 20th century [1], and this observation was confirmed in subsequent publications [2, 3]. Subepithelial fibrosis, a characteristic feature of asthma, is a result of tenascine, fibronectine, proteoglicanes and collagen type I, III and V deposition in lamina densa of airway basement membrane [4–7]. Following the first description of subepithelial fibrosis of asthmatic airways by Roche et al. in 1990 [4], airway wall remodeling has been subject to extensive research. Our knowledge of chronic inflammation and airway structural changes in asthma is based on bron-

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choscopic studies (bronchoalveolar lavage fluid, bronchial mucosal biopsies) and high resolution computed tomography (HRCT) [8–10].

Structural abnormalities referred to as airway remodeling include: epithelial desquamation, BM thickening, hypertrophy of the goblet cells and mucous glands, increased proliferation of blood vessels, hyperplasia and smooth muscle cell hypertrophy [11–13]. The thickening of whole bronchial wall is a consequence of bronchial remodeling [10, 14]. It has been suggested that airway remodeling is the main reason for airway hyper-responsiveness, exaggerated loss of lung function or the irreversible bronchial obstruction observed in some asthma patients, the disease taking a more severe course, or resistance to glucocorticosteroid therapy [15].

We felt that analysis of the relationship between the extent of airway remodeling and lung function parameters could be helpful in assessing the pathophysiological consequences of airway wall remodeling. Moreover, the results of several studies assessing the consequences of bronchial remodeling are inconsistent [4, 16]. The main aim of our study was to assess the relationship between lung function parameters and BM thickness in patients with asthma.

Material and methods

Twenty patients with asthma (ten men and ten women aged between 18 and 76 (mean 37 ± 15 years) were included. The mean duration of symptoms was 14.5 ± 13 years. The diagnosis of asthma and the assessment of disease severity were performed in accordance with the Global Strategy for Asthma Management and Prevention guidelines [17]. Intermittent asthma was diagnosed when symptoms were infrequent (less than once a week) and nocturnal symptoms less than twice a month with $FEV_1 \geq 80\%$ pred. Mild asthma was defined in patients with symptoms more than once a week but less than once a day, with nocturnal dyspnea more than twice a month with $FEV_1 \geq 80\%$ pred. Patients with moderate asthma had symptoms once daily and more than once during the night with FEV_1 between 60–80% pred.

Study inclusion criteria were: intermittent, mild or moderate asthma and no glucocorticosteroid therapy for at least three months before enrollment.

In ten patients, asthma was diagnosed just before the enrollment (this was called the asthma newly recognized or AN group). In the others, the time between diagnosis of the disease and study onset was between two and 24 years (this was called the asthma recognized years before the study or AD group) (mean 12.4 ± 7.9 years). There were 12 (60%) patients with allergic asthma, and seven (35%) patients had chronic allergic rhinitis. There were 11 never-smokers, and six ex-smokers (they stopped smoking at least two years before the study). Three patients were current smokers at the time of the study (mean 6 ± 12 pack-years). All patients had not been treated with glucocorticosteroids for at least three months before the study onset.

This study was part of a research project approved by the Bioethics Committee of the Medical University of Warsaw, Poland (approval No. 172/2003). All patients provided written informed consent.

All patients underwent extensive clinical evaluation including medical history and physical examination, chest X-ray, lung function testing, arterial blood gas analysis, skin prick tests (Allergopharma, Germany) and basic biochemistry panel including total serum IgE. Lung function testing comprised flow-volume curve (Lungtest 1000, MES, Poland) according to European Respiratory Society standards [18], body plethysmography with the measurement of lung volumes, bronchial resistance and diffusion capacity for carbon monoxide (Vmax Series 229/V6200, Sensor Medics Corporation, Yorba Linda, USA) and metacholine challenge in accordance with the ATS guidelines [19]. Obstruction reversibility test (salbutamol 200 μg) was interpreted in accordance with Polish Pneumonology Society guidelines [20].

There were two (10%) patients with intermittent asthma, nine (45%) with mild persistent and nine (45%) with moderate persistent disease. Mean value of forced expiratory volume in first second (FEV_1% pred.) was 82.6 ± 17.8%. Other lung function tests results are presented in Table 1.

All patients underwent bronchofiberoscopy as previously described [21]. Fiberoptic bronchoscopy (11004 BC, Storz, Germany) was performed under local anaesthesia (2% lidocaine) after premedication with atropine sulphate 0.5 mg IM, diazepam 10 mg IM and inhaled salbutamol 400 μg. After visual inspection of the lower airways, between two and four forceps mucosal biopsies were taken from the segmental bronchi of the middle lobe or lower lobes.

The specimens were fixed in 4% buffered formaldehyde solution and routinely processed to paraffin blocks. Four-μm-thick sections were stained with haematoxylin and eosin (H & E) and used to evaluate basement membrane thickness (BMT) and the epithelium. The slides were assessed by light microscopy (Olympus, Japan) at × 400 ma-

Table 1. Results of lung function tests

Lung function tests results	Mean ± SD
FEV_1% VC	69 ± 9
FEV_1% predicted	83 ± 18
FVC% predicted	101 ± 14
TLC% predicted	107 ± 12
RV% predicted	127 ± 44
RV/TLC	34 ± 13
Raw [cm H_2O/l/s]	2.6 ± 1.3
Obstruction reversibility [ml]	358 ± 193
PC_{20} [mg/ml]	2.5 ± 3.1

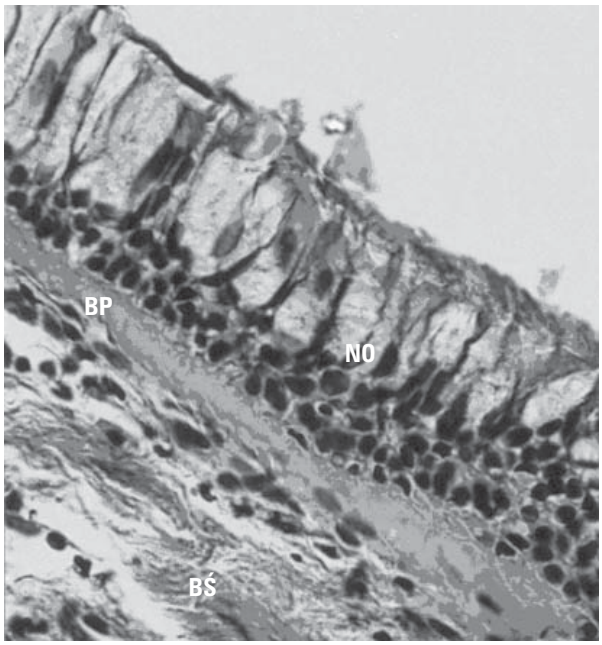

Figure 1. Mucosa specimen collected from a patient with moderate asthma. BP — reticular basement membrane, NO — normal epithelial bronchial layer, BŚ — mucosa propria. Light microscope (magnification × 400)

gnification (× 40 objective lens, × 10 eyepiece). Only sections perpendicular to the epithelial surface and the BM were selected for measurement (Figure 1).

Computer software MultiScan Base 08.98 CSS Video Frame Grabber v.5.10 (Computer Scanning Systems, Poland) was used to measure basement membrane thickness (BMT). At least 40 measurements at 20 μm intervals were taken in each patient in accordance with the method developed by Sullivan et al. [22]. Two independent pathologists, blinded to patients' diagnoses, were involved in the evaluation of the biopsy specimens. The mean number of collected biopsy specimens was 3 ± 1 per patient. The mean number of BMT measurements was 53 ± 16 per patient.

All statistical calculations were performed using Statistica 6.0 (StatSoft Inc., USA). Numeric values were presented as mean ± standard deviation. Ranges were also provided for selected variables. The Mann-Whitney U-test and the Kruskal-Wallis ANOVA test were applied to compare two or more unrelated samples, respectively. Spearman's rank correlation coefficient was applied to test potential correlations between different variables. P values below 0.05 were considered statistically significant, other p values were described as p = ns (not significant).

Results

The mean basement membrane thickness (BMT) was 12.8 ± 2.8 μm (min. 8.5 μm, max. 20.7 μm). No relationship between BMT and the duration of the disease or the patient's age were revealed. There was also no correlation between BMT and asthma severity. The mean BMT was 15.3 ± ± 1.2 μm, 11.8 ± 2.2 μm and 13.2 ± 3.3 μm in patients with intermittent, mild and moderate asthma, respectively (p = ns). There were no statistical differences between BMT in patients whose asthma had lasted more than five years and the group with asthma duration of less than five years (13.2 ± 3.1 μm, and 12.1 ± 1.8 μm; respectively, p = ns). In the AN group, BMT was 12.3 ± 1.9 mm. In the AD group, it was 13.3 ± 3.3 mm, ns.

The mean BMT in patients with atopy was comparable with the mean BMT in non-atopic subjects (12.6 ± 2.0 μm vs 13.0 ± 3.6 μm, respectively, ns). In allergic rhinitis patients, the BMT was slightly higher than in patients without rhinitis, but the difference was not statistically significant (13.6 ± ± 1.0 μm *vs.* 12.4 ± 3.0 μm, p = ns).

There was no relation between BMT and FEV_1% pred., FVC% pred. and RV% (residual volume), TLC% (total lung capacity) and Raw (airway resistance) pre- as well as post-bronchodilator. No relationship was found between basement membrane thickness and airway responsiveness referred to as PC_{20} value (provocative concentration).

Discussion

Airway remodeling and its consequences are subject to extensive research. Considering the fact that airway remodeling is a complex process, it is difficult to assess several pathophysiological and clinical results of bronchial structural changes.

Table 2. Airway basement membrane thickness (μm) in healthy and asthma patients. Results of several studies

Author	Healthy (n)	Asthma (n)	Microscopy
Roche 1989 [4]	4.17 ± 0.59 (3)	7.95 ± 1.79 (8)	ME
Ollerenshaw 1992 [25]	—	12 ± 2 (10)	ML
Jeffery 1992 [6]	8.2 ± 1.7 (12)	11.25 ± 2.9 (11)	ML
Trigg 1994 [26]	—	23.13 ± 3.44 (12)	ME
O'Shaughnessy 1996 [27]	4.3 ± 0.4 (5)	8.3 ± 0.6 (12)	ML
Milanese 2001 [28]	—	10.1 ± 3.7 (11)	ML
Payne 2003 [30]	Adults 4.4 (8) Children 4.9 (10)	Adults 8.1 (10) Children 8.2 (19)	ML
Köksal 2005 [31]	4.1 ± 1.7 (8)	—	ML

Mean values of airway basement membrane in micrometers are presented ± SD, n — number of patients, ME — electron microscopy, ML — light microscopy

Bronchofiberoscopy with bronchial biopsies is a common method used in the assessment of airway remodeling in asthma patients. It was introduced to assess airway remodeling in asthma more than 20 years ago [23]. In our study, bronchofiberoscopy was well tolerated and there were no substantial respiratory or circulatory complications [21]. The safety of bronchoscopy performed in asthmatic patients has been well documented [24].

In our group of asthma patients, airway BMT was 12.8 ± 2.8 μm. This appears consistent with BMT values assessed by other authors. However, it must be noted that there are some differences in BMT assessment between the studies. The results of some studies suggest that BMT in asthma patients could range from 7.9 μm to 23 μm [4, 6, 25––28]. Considering these findings, subepithelial membrane dimension can be related to bronchoscopy technique, the fixation and staining methods and the measurement undertaken in oblique-cut sections [29]. Therefore, all bronchoscopies in our study were performed according to the same protocol, accounting for the operator, type of biopsy forceps, as well as the method of fixation and staining of the specimens. The measurements were taken only in sections perpendicular to the epithelial surface with well-visualized basement membrane and epithelium (Figure 1) according to the technique described as the most accurate in BMT measurement [22].

Basement membrane thickening in the course of asthma has long been recognized. It has been also indicated in several studies that BM in asthmatics is much thicker than in healthy subjects [4, 27, 30]. According to several reports, mean basement membrane thickness in healthy subjects is estimated to be between 2.4 and 9.9 μm (Table 2) [4, 25, 27, 30–32].

Comparing the BMT assessed in other studies with our findings (mean BMT 12.8 ± 2.8 μm), it can be assumed that airway basement membrane in our patients was considerably thickened. This thickening was observed in patients with mild and moderate asthma as well as intermittent asthma. There was no difference between the three groups in terms of BMT. Our findings are consistent with the results of other studies [33, 34].

Our study has some limitations. The study group was relatively small, although comparable to some other studies (Table 2). The main limiting factors affecting sample size were patient consent to bronchoscopy and the requirement not to use glucocorticosteroids in the pre-study period.

Even though some studies suggest that the extent of airway remodeling relates to the duration and severity of asthma [35, 36], the results of our study are consistent with observations of other groups that BMT is unrelated to the age of the patient, duration of asthma or its severity [30, 37, 38].

Structural changes have been found in patients with mild asthma, treated or not treated with inhaled glucocoricosteroids [39]. They have been observed even in well-controlled asthma [40]. Thickening of basement membrane has been found in patients years before asthma was diagnosed [41] and in patients with allergic rhinitis [28] or atopy [42], without diagnosis of asthma at the time of assessment.

It should be noted that our asthmatic patients had not been treated with corticoids for at least three months before study enrollment. In our opinion, this was obligatory, as there were observations of the influence of inhaled steroid therapy on airway remodeling (including basement membrane dimension) [6, 8, 39].

Since it was observed that in some asthmatic patients, like in COPD, airway obstruction can become irreversible, plenty of opinions have been expressed that bronchial remodeling can be the reason. Some authors link bronchial structural changes with airway hyper-responsiveness and a more severe course of the disease [43]. But the relation between airway structural changes and lung function parameters is not consistent. Our study found no correlation between BMT and lung function test results including FEV_1% pred., RV% pred. and Raw.

One of the fist descriptions of the correlation between BMT and limitation of airway airflow (referred to as diminished FEV_1) was the study of Kasahara et al. They observed a relationship between BMT and post-bronchodilator FEV_1% pred. in a group of 22 asthmatics [9]. Other studies also suggest the relationship between whole airway wall thickness and the degree of bronchial obstruction [39]. Benayoun et al. found that airway obstruction can be related to the number of fibroblasts found under the basement membrane and smooth muscle cell hypertrophy in the airway wall [38]. Minshall et al. observed that the thicker the basement membrane, the smaller the FEV_1% pred. [44]. In some other studies of patients with asthma, a negative correlation between BMT and FEV_1% pred. was also found [35, 39].

On the other hand, there are observations that in children with severe asthma there is no correlation between basement membrane dimension and FEV_1% pred. pre- and post-bronchodilator [45]. Payne et al. did not find a relationship between BMT and FEV_1% pred. and concentration of exhaled nitric oxide (some researchers assume that this could be the marker of remodeling as well as airway inflammation) in children with asthma [30]. Similarly, there was no relation between bronchial obstruction and BMT in the study of Boulet et al. in adults [47] and of Jenkins et al. of six children with severe asthma [48].

Slight basement membrane thickening has been observed in patents after lung transplantation, but in this group there was no correlation between BMT and FEV_1 value [49]. On the other hand, Ward et al., indirectly by the measurement of airway distensibility, found that airway obstruction may be related to basement membrane dimension [50].

The discrepancies in the results of studies assessing the correlations between remodeling and lung function test could be explained by the differences in glucocorticosteroid therapy regime. Ward et al. revealed that the extent of airway remodeling e.g. basement membrane thickening and lung function test results differ during the course of glucocorticoid therapy [51]. The lack of relation between FEV_1 and BMT does not mean that remodeling has no influence on lung function at all. It seems rather that airway obstruction in asthmatic patients is considerably related not only to basement membrane thickening but also to other components of remodeling not assessed in our study. Some publications suggest that, in asthma, airway obstruction [52] and irreversible bronchial obstruction [53] are mainly associated with muscle hypertrophy. We did not assess this in our study.

Besides airway remodeling, several other factors in asthma may result in airflow limitation and airway obstruction: airway constriction, inflammatory oedema of bronchial mucosa or mucus in airway lumen. Patients included in the study were not treated with glucocorticosteroids. It is probable then, that besides remodeling, the degree of bronchial obstruction could be the result of some reversible changes such as oedema of bronchial mucosa.

The relation between airway reactivity and remodeling is also uncertain. There are studies suggesting the relationship between basement membrane thickness and airway reactivity [39, 47, 54]. On the other hand, Boulet et al. found a correlation between hyper-responsiveness and remodeling assessed as whole bronchial wall thickening but only in patients with irreversible obstruction [55]. Boulet et al. [16] and Jeffery et al. [56] observed a relation between subepithelial fibrosis and airway hyper-responsiveness in the group of patients with mild asthma. Our study did not find any relationship of BMT and airway responsiveness referred to as PC_{20} and our findings are consistent with the results of Roche et al. [4]. Some works suggest that bronchial remodeling can prevent exaggerated airway smooth muscle constriction. Milanese et al. observed that the thicker the basement membrane, the higher the dose of metacholine needed to achieve a positive hyper-responsiveness test [28].

Conclusions

The assessment of airway wall remodeling and its clinical consequences is difficult and the data from several studies is inconsistent. The results of our study may suggest that the thickening of basement membrane does not depend on the duration of asthma or its severity or the age of the patient. In our study there was no relation between airway basement membrane and lung function parameters in patients with intermittent, mild and moderate asthma in stable disease.

The results of our study suggest there is no relation between basement membrane and airway responsiveness in asthmatic patients. And that thickening of basement membrane alone does not result in a limited airflow in airways of asthma patients.

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