Remodeling of basement membrane in patients with asthma

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Abstract
The “bronchial remodeling” specific for the asthmatic disease consists in irreversible changes of the bronchial wall, including glandular and smooth muscle fibers hyperplasia and/or hypertrophy, goblet cells hyperplasia, and thickening of basement membrane (BM). We aimed to analyze the BM thickness in asthma patients, in order to validate the relationship between its changes and the disease severity defined in agreement with the Global Initiative for Asthma (GINA) criteria. The study group has been formed of 38 patients with different degrees of severity of asthma established by spirometry using Forced Expiratory Volume in one second (FEV1), and two patients without asthma symptoms as controls. The specimens harvested by fibrobronchoscopy have been processed by paraffin embedding followed by Hematoxylin–Eosin (HE) and Periodic Acid–Schiff (PAS) staining. For each case, the BM measurement has been realized by a “point-by-point” method. Statistical analysis has been performed using SPSS 17 software, by applying non-parametric correlation tests. The quantitative assessment revealed a progressive increase in BM thickness during the course of the disease, from a mean value of 11.2 μm in stage 1 to that of 15.6 μm in stage 4. Even if this process has been noticed starting with the first stage of asthma, the differences in the BM size were statistically significant only for stages 1 and 3 (p=0.047), stages 1 and 4 (p=0.000), stages 2 and 3 (p=0.000), and stages 3 and 4 (p=0.000). Spearman’s test has shown an opposite correlation between the BM thickness and asthma severity defined by FEV1 values (r=−0.86, p<0.01, 95% CI). Our study demonstrates that the collagen deposition at the epithelium-connective interface is initiated in early stages of asthma and continues in a progressive modality, the BM thickening being correlated with the disease severity. Thus, we support the concept of biological consequences of BM thickening in asthma pathogenesis, a mechanism still incompletely deciphered.

Keywords: basement membrane, bronchial remodeling, asthma.

Introduction
Asthmatic disease is characterized by a chronic mucosal inflammatory process, which results in irreversible changes of the bronchial wall, known today as “bronchial remodeling” [1–3]. Glandular and smooth muscle fibers hyperplasia and/or hypertrophy, goblet cells hyperplasia, and variable thickening of basement membrane (BM) present under the respiratory epithelium are part of these morphological changes [4–6]. Normal thickness of BM is 5–6 μm, while literature reports range up to ×2–3 folds increase in all patients diagnosed with bronchial asthma [7]. This thickening, considered as a marker of the remodeling process characteristic for this disease [7–9], occurs due to excess deposition of collagen type I in lamina reticularis of the BM, as a result of T-cells and subepithelial myofibroblasts activation [9, 10]. Functionally, lamina reticularis thickening is related to airways reduced distensibility, suggesting a negative impact on pulmonary airflow. Consequently, the changes generated at the epithelium–connective interface account for an “adaptive response” to inflammatory stress and sporadic bronchoconstriction [11–14]. However, current data on BM reactivity in asthmatic patient are still incomplete for an accurate assessment of its involvement in pathogenesis and specifically in bronchial wall remodeling, mainly as collagen deposits in lamina reticularis are not correlated to the degree of disease severity [7, 15, 16].

The present study supplements our previous research focused on the structural modifications in correlation with asthma severity [17]. Starting from clinical data, we aimed to analyze the BM thickness in asthma patients, in order to validate the relationship between its changes and the severity of the disease established in agreement with the Global Initiative for Asthma (GINA) criteria.

Materials and Methods
The study group consisted of 38 patients diagnosed with asthmatic disease in the Specialty Ambulatory of the Pneumology Clinic Hospital, Iassy, Romania. Two patients without asthma symptoms, investigated for tumor suspicion, which has been later dismissed, represented the control group.

The study protocol had the approval of the Ethics Committee of the Pneumology University Hospital and “Grigore T. Popa” University of Medicine and Pharmacy, Iassy.

The inclusion of patients within the studied group had been based on informed consent and on well-defined criteria: non-smokers (total non-smoker or former occasional smoker), absence of any other acute or chronic pulmonary disease, capacity to correctly perform a spirometry, and consent for fibrobronchoscopy with biopsy. The exclusion criteria have been: smokers, patients refuse to be investigated by fibrobronchoscopy, relative contraindications for...
fibrobronchoscopy (advanced age, coagulopathies, liver and/or renal failure, allergies, and severe heart conditions).

After anamnesis, patients have been initially investigated by thoracic radiography and pulmonary functional test [spirometry evaluation using Forced Expiratory Volume in one second (FEV1)], in order to establish the severity degree of asthma, according to GINA criteria [18]. Subsequently, fibrobronchoscopic examination has been performed using the video endoscope camera Olympus BF Type 160, with biopsies of the bronchial mucosa from the lobar median or right lobar inferior prominences. In order to reduce the risk of insufficient or inconclusive biopsy to a minimum, 5–7 tissue fragments have been taken for each patient, according to international regulations [19, 20].

The tissue fragments have been fixed for 24 hours in 10% formalin and paraffin embedded. Serial sections of 4 μm have been cut, dewaxed and stained with Hematoxylin–Eosin (HE) and Periodic Acid–Schiff (PAS).

The examination of HE-stained slides had allowed selection of biopsies that fulfilled the following quality standards: correct fixation and staining, sufficient connective tissue subjacent the respiratory epithelium (minimum 0.3 mm), lack of degenerated tissue, mucus, extensive hemorrhage or hyaline cartilage [21].

For each case, the basement membrane assessment has been performed on a single slide, chosen by the proper quality of the tissue content. Two independent pathologists have accomplished multiple measurements („point-by-point”) at 20 μm intervals, for 1 mm length, excluding the territories that had inadequate orientation, using ×200 magnification [22]. All data have been achieved by Lucia Net software and Sony DN 100 camera connected to Nikon Eclipse E600. Finally, a mean value per case, based on all measurements, has been calculated.

Statistical analysis has been performed using SPSS 17 software, by applying non-parametric correlation tests (Newman–Keuls and Spearman).

Results

Following clinical examination and pulmonary exploratory tests (Table 1), the patients have been included into the following stages: seven cases of intermittent bronchial asthma (stage 1), 10 cases of slightly persistent bronchial asthma (stage 2), 16 cases of moderately persistent bronchial asthma (stage 3), and five cases of severely persistent bronchial asthma (stage 4).

The qualitative microscopic exam of HE-stained specimens revealed an increased BM thickness of bronchial lining epithelium in all asthma patients included in our study. This feature has been noticed from the intermittent stage of the disease, BM thickness being more obvious as the patient was placed in a higher stage of disease severity (Figures 1 and 2).

<table>
<thead>
<tr>
<th>Bronchial asthma stages</th>
<th>No. of cases</th>
<th>FEV1 (% of ideal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent – stage 1</td>
<td>7</td>
<td>106.3±14</td>
</tr>
<tr>
<td>Slightly persistent – 2</td>
<td>10</td>
<td>94.5±6.3</td>
</tr>
<tr>
<td>Moderately persistent –</td>
<td>16</td>
<td>66.7±6.2</td>
</tr>
<tr>
<td>Severely persistent – 4</td>
<td>5</td>
<td>43.6±10.8</td>
</tr>
</tbody>
</table>

FEV1: Forced Expiratory Volume in one second; SD: Standard deviation; Q25: Lower quartile; Q75: Upper quartile.

The quantitative assessment of the BM thickness has led to the following data:

- in intermittent bronchial asthma, BM mean value is 11.2±1.0 μm, with a minimum of 9.8 μm and a maximum of 12.4 μm;
- in slightly persistent bronchial asthma, BM mean value is 11.6±0.9 μm, with a minimum of 9.9 μm and a maximum of 12.7 μm (Figure 3a);
- in moderately persistent bronchial asthma, BM mean value is 12.4±0.7 μm, with a minimum of 11.5 μm and a maximum of 13.4 μm (Figure 3b);
- in severely persistent bronchial asthma, BM mean value is 15.6±0.7 μm, with a minimum of 14.8 μm and a maximum of 16.4 μm (Figure 3c);
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Figure 3 – Different aspects of the BM thickening in (a) intermittent bronchial asthma, (b) moderate persistent bronchial asthma, (c) severe persistent bronchial asthma. PAS staining, ×200.

All results of BM measurement on PAS-stained specimens, for all cases corresponding to the four stages of severity, are presented in Table 2. In controls, the median thickness of the BM had 5.3 μm (Figure 4).

Table 2 – Quantitative assessment of basement membrane patients included in the study group

<table>
<thead>
<tr>
<th>Intermittent bronchial asthma</th>
<th>Case No. / BM thickness [μm]</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>10.3</td>
<td>12.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Slightly persistent bronchial asthma</th>
<th>Case No. / BM thickness [μm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>11.9</td>
<td>12.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Moderately persistent bronchial asthma</th>
<th>Case No. / BM thickness [μm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>12.2</td>
<td>11.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Severely persistent bronchial asthma</th>
<th>Case No. / BM thickness [μm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>16.4</td>
<td>15.5</td>
</tr>
</tbody>
</table>

BM: Basement membrane.

Statistical analysis performed by Newman–Keuls test has revealed statistical differences between the studied groups defined in relation to asthma severity degree according to FEV1 and BM thicknesses, as follows: intermittent versus moderately persistent stage ($p=0.047$), intermittent versus severely persistent stage ($p=0.000$), slightly persistent versus severely persistent stage ($p=0.000$), and moderately persistent versus severely persistent stage ($p=0.000$). No significant differences have been registered for intermittent bronchial asthma versus slightly persistent bronchial asthma ($p=0.862$), neither in slightly persistent bronchial asthma when compared to moderately persistent asthma ($p=0.1117$). Additionally, Spearman’s test has shown an opposite correlation between the BM thickness and asthma severity defined by FEV1 values ($r=-0.86, p<<0.01, 95\% CI$).

Figure 4 – Normal BM in control. PAS staining, ×200.

Discussion

Despite the progress registered in functional investigations of asthmatic disease, the most accurate information regarding the severity of lesions is founded on the morphological appearance of bronchial biopsies obtained...
by fibrobronchoscropy [23, 24]. Unfortunately, a relatively reduced number of studies report bronchial wall alterations, because fibrobronchoscropy – with or without biopsy – is not a current procedure in asthmatic disease [19].

Within this context, although the modification of BM has been one of the first structural changes detected in the remodeling process of the bronchial wall in asthmatic patients [1, 7, 9, 25, 26], the exact relationship between this alteration and the disease severity is incompletely known. Therefore, the investigation of the BM is still a matter of interest, considering both its size and the overexpression of its molecular components [2, 27–29].

Our study allowed the quantification of the BM thickness in asthmatic patients of different degrees of disease severity, according to GINA 2014, by microscopic analysis of tissue fragments obtained by fibrobronchoscopic exam. The measurements revealed a progressive increase in BM thickness during the course of the disease, from a mean value of 11.2 μm in stage 1 to that of 15.6 μm in stage 4. Even if this process has been noticed starting with the first stage of asthma, the differences in the BM size were significant only for stages 1 and 3 (p = 0.047), stages 1 and 4 (p = 0.000), stages 2 and 3 (p = 0.000), and stages 3 and 4 (p = 0.000). No statistically significant differences have been recorded between stages 1 and 2, and stages 2 and 3 (p = 0.862, p = 0.1117, respectively). All these data show that the collagen deposition at the epithelium–connective interface occurs in early stages of the disease [25] and continues in a progressive manner. The mechanism could be explained by the activation of T-cells and myofibroblasts that act as an amplification loop, each inflammatory cycle being more enhanced than the previous and less enhanced than the next stage.

As we have already mentioned above, there is a limited number of researches dedicated to the correlation between BM thickness and asthma stages, due to the difficulty to perform fibrobronchoscopy in asthmatic patients [22, 30–34]. However, our measurements correspond to the published results summarized below. In a study performed on 34 patients (14 with early, 14 with moderate, and six with severe asthma) and eight controls [33], the BM measurements showed a general mean value of 12.4±3.3 μm compared to 4.4±0.5 μm in controls, with the following detailed values: 10.8±2.4 μm in early type, 12.1±2.7 μm in moderate type, and 16.7±3.1 μm in severe type [33]. BM thickening is not related to age, duration of the disease or patients gender, as demonstrated by a study performed on 18 children diagnosed with bronchial asthma and 24 controls, where the affected BM had a mean value of 8.3±1.4 μm in patients, whereas the mean value was 6.8±1.3 μm in normal status [34]. Moreover, a two-fold analysis, in light and electron microscopy [32], performed on biopsies from 15 asthmatic patients and 13 controls, led to comparable results – namely BM thickness of 6.0±2.09 μm in asthma and 3.19±0.55 μm in controls, by light microscopy, and 6.09±2.19 μm and 2.85±0.86 μm, by electron microscopy.

A short remark on methodology is compulsory. Despite the similarities of BM thickness reported results, they have been obtained through different measurement methods [22, 30–34]. In this respect, numerous controversies have been initiated by different modalities of BM measurement, without any conflict of interests, funding and other personal relationship with other people or organizations related to this study.

**Conclusion**

Our data demonstrate that the collagen deposition at the epithelium–connective interface is initiated in early stages of asthma and continues in a progressive modality, the BM thickening being correlated with the disease severity. Thus, we support the concept of biological consequences of BM thickening in asthma pathogenesis, a mechanism still incompletely deciphered.

**Conflict of interests**

The authors deny any conflict of interests, funding and other personal relationship with other people or organizations related to this study.

**References**


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