ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Remodeling of basement membrane in patients with asthma

ADRIANA GRIGORAŞ[1,2)], CONSTANTIN CRISTIAN GRIGORAŞ[3)], SIMONA ELIZA GIUŞCĂ[1)], IRINA-DRAGA CĂRUNTU[1)], CORNELIA AMĂLINEI[1,2)]

[1)]*Department of Morphofunctional Sciences, "Grigore T. Popa" University of Medicine and Pharmacy, Iassy, Romania*

[2)]*Department of Histopathology, Institute of Legal Medicine, Iassy, Romania*

[3)]*Specialty Ambulatory, Pneumology Clinic Hospital, Iassy, Romania*

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 ha 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 1 – ***Functional pulmonary parameters – patients included in the study group***

Bronchial asthma stages	No. of cases	FEV1 (% of ideal)					
		Mean values ± SD	*Minimum*	*Q25*	*Median*	*Q75*	*Maximum*
Intermittent – stage 1	7	106.3±14	88.0	92.8	111.0	119.0	122.0
Slightly persistent – stage 2	10	94.5±6.3	82.0	92.0	93.7	100.0	103.0
Moderately persistent – stage 3	16	66.7±6.2	59.2	62.0	64.5	70.3	79.8
Severely persistent – stage 4	5	43.6±10.8	25.3	45.0	45.2	48.8	53.7

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

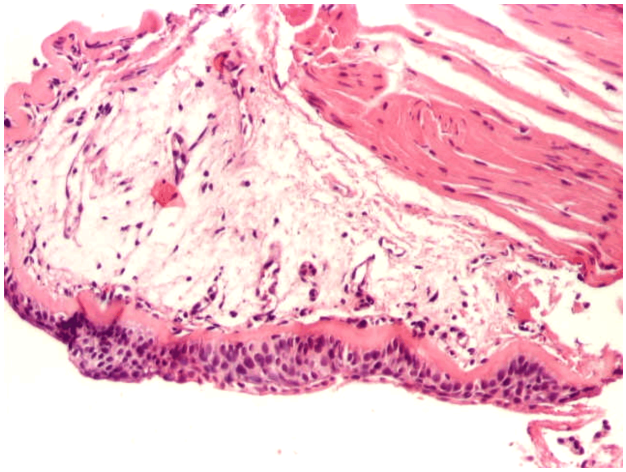

Figure 1 – ***Slightly persistent bronchial asthma – evident thickness noted at the epithelium–connective interface. HE staining, ×100.***

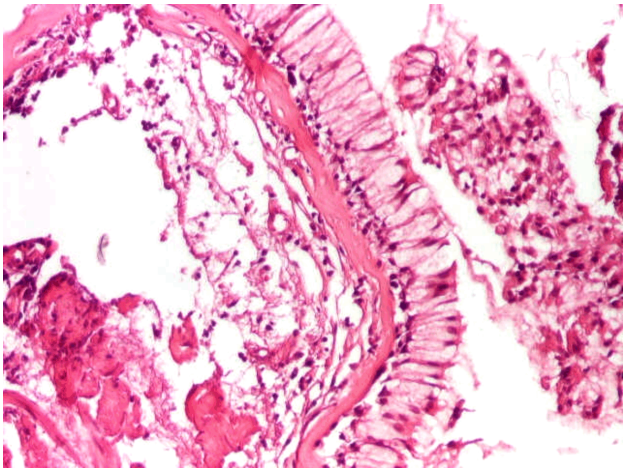

Figure 2 – ***Moderately persistent bronchial asthma – marked thickness registered at the epithelium–connective interface, goblet cell hyperplasia. HE staining, ×100.***

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);

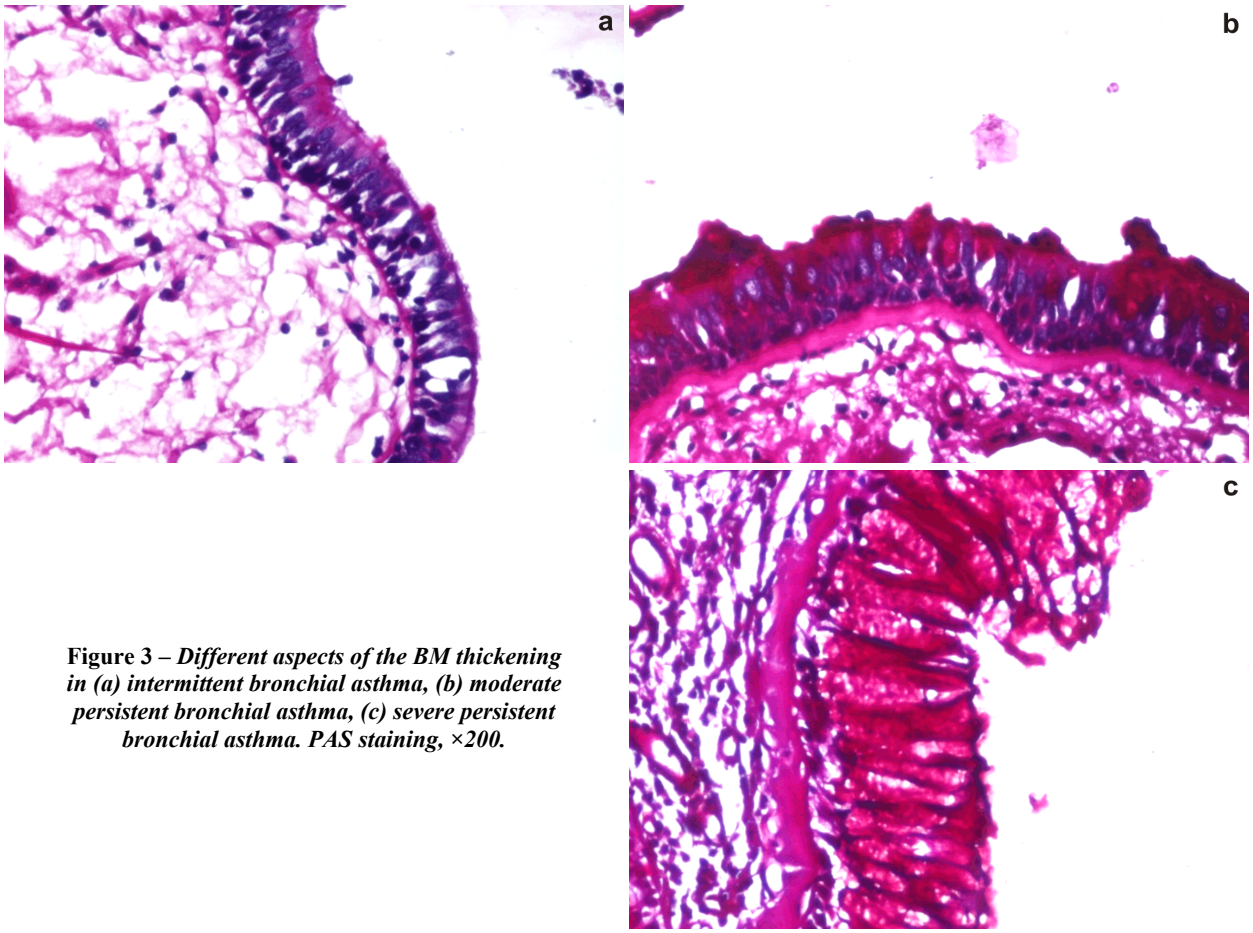

Figure 3 – ***Different aspects of the BM thickening in (a) intermittent bronchial asthma, (b) moderate persistent bronchial asthma, (c) severe persistent bronchial asthma. PAS staining, ×200.***

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***

Intermittent bronchial asthma									
Case No. / BM thickness [μm]									
1	*2*	*3*	*4*	*5*	*6*	*7*			
10.3	12.4	12.1	11.8	10.9	9.8	11.2			
Slightly persistent bronchial asthma									
Case No. / BM thickness [μm]									
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
11.9	12.5	11.6	10.2	9.9	11.7	10.9	12.3	11.8	12.7
Moderately persistent bronchial asthma									
Case No. / BM thickness [μm]									
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
12.2	11.7	12.4	12.8	11.7	11.5	13.3	12.6	11.9	11.6
11	*12*	*13*	*14*	*15*	*16*				
12.7	12.9	13.2	12.8	11.5	13.4				
Severely persistent bronchial asthma									
Case No. / BM thickness [μm]									
1	*2*	*3*	*4*	*5*					
16.4	15.5	16.2	14.8	15.1					

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).

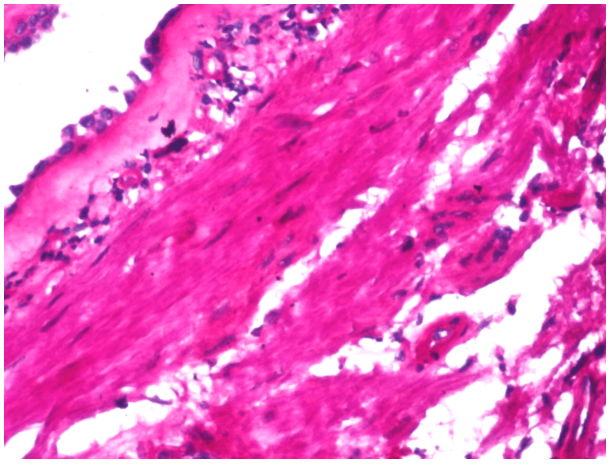

Figure 4 – ***Normal BM in control. PAS staining, ×200.***

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 fibrobronchoscopy [23, 24]. Unfortunately, a relatively reduced number of studies report bronchial wall alterations, because fibrobronchoscopy – 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 one.

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 6.01±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 quantification standardized method consensus. The most used methods are Sullivan method [22], used also in our study, and Wilson and Li method [32]. While the method proposed by Sullivan uses at least 40 measurements ("point-by-point") at regular intervals of 20 μm, for each fragment of bronchial mucosa, stained by special methods or by immunohistochemistry, Wilson and Li method [32] requires the measurement on the entire biopsy length, in HE staining – a more difficult procedure, which raises debates about the results accuracy. However, regardless of the applied measurement method, the accurate assessment of BM thickening firstly involves an accurate histological method of handling the specimen in order to avoid or to minimize the fixation, staining, or orientation artifacts and, not the least, a study of a large cohort [21].

The results of our study contribute to a better knowledge of the morphological lesions developed in asthma, in correlation to the disease progression. Concomitantly, large perspectives are opened for a more targeted therapy. It is already well known that high dosage corticoids used in therapeutic scheme in severe persistent type of disease seem to be beneficial in BM thinning [16]. Consequently, the constant interest on the BM thickness is strongly supported by the possibility to design and implement new therapeutic approaches, that intend to prevent or, at least, to reduce the amplitude of the lesions produced at the epithelium–connective junction.

Conclusions

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

[1] Sumi Y, Hamid Q. Airway remodeling in asthma. Allergol Int, 2007, 56(4):341–348.
[2] Jeffery PK. Remodeling and inflammation of bronchi in asthma and chronic obstructive pulmonary disease. Proc Am Thorac Soc, 2004, 1(3):176–183.
[3] Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med, 2000, 161(5): 1720–1745.
[4] Gras D, Chanez P, Vachier I, Vachier I, Petit A, Bourdin A. Bronchial epithelium as a target for innovative treatments in asthma. Pharmacol Ther, 2013, 140(3):290–305.
[5] Grainge CL, Lau LC, Ward JA, Dulay V, Lahiff G, Wilson S, Holgate S, Davies DE, Howarth PH. Effect of bronchoconstriction on airway remodeling in asthma. N Engl J Med, 2011, 364(21):2006–2015.
[6] Shifren A, Witt C, Christie C, Castro M. Mechanisms of remodeling in asthmatic airways. J Allergy (Cairo), 2012, 2012:316049.
[7] Sładek K. Remodelling in obturative diseases and treatment. Pol Merkur Lekarski, 2010, 29(172):227–230.

[8] Bergeron C, Tulic MK, Hamid Q. Airway remodelling in asthma: from benchside to clinical practice. Can Respir J, 2010, 17(4):e85–e93.
[9] Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. Lancet, 1989, 1(8637): 520–524.
[10] Trejo Bittar HE, Yousem SA, Wenzel SE. Pathobiology of severe asthma. Annu Rev Pathol, 2015, 10:511–545.
[11] Holgate ST. Innate and adaptive immune responses in asthma. Nat Med, 2012, 18(5):673–683.
[12] James AL, Maxwell PS, Pearce-Pinto G, Elliot JG, Carroll NG. The relationship of reticular basement membrane thickness to airway wall remodeling in asthma. Am J Respir Crit Care Med, 2002, 166(12 Pt 1):1590–1595.
[13] Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. Am J Respir Cell Mol Biol, 1990, 3(5):507–511.
[14] Holgate ST, Arshad HS, Roberts GC, Howarth PH, Thurner P, Davies DE. A new look at the pathogenesis of asthma. Clin Sci (Lond), 2009, 118(7):439–450.
[15] Kudo M, Ishigatsubo Y, Aoki I. Pathology of asthma. Front Microbiol, 2013, 4:263.
[16] Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. Am J Respir Crit Care Med, 2003, 167(10):1360–1368.
[17] Grigoraş A, Căruntu ID, Grigoraş CC, Mihăescu T, Amălinei C. Relationship between immunohistochemical assessment of bronchial mucosa microvascularization and clinical stage in asthma. Rom J Morphol Embryol, 2012, 53(3):485–490.
[18] Global Initiative for Asthma (GINA) Management and Prevention. National Institute of Health. National Heart, Lung and Blood Institute, GINA, NHLBI/World Health Organization (WHO), 2014. Available from: http://www.respiratoryguidelines.ca/GINA-guidelines-resources.
[19] Ulmeanu R, Mihălţan FD, Cordoş I, Ulmeanu DI. Actualităţi în endoscopia toracică diagnostică şi terapeutică [in Romanian]. Ed. Universitară "Carol Davila", Bucureşti, 2009, 51–150.
[20] Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS, GOLD Scientific Committee. Global strategy for diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. Am J Respir Crit Care Med, 2001, 163(5):1256–1276.
[21] Elston WJ, Whittaker AJ, Khan LN, Flood-Page P, Ramsay C, Jeffery PK, Barnes NC. Safety of research bronchoscopy, biopsy and bronchoalveolar lavage in asthma. Eur Respir J, 2004, 24(3):375–377.
[22] Sullivan P, Stephens D, Ansari T, Costello J, Jeffery P. Variation in the measurements of basement membrane thickness and inflammatory cell number in bronchial biopsies. Eur Respir J, 1998, 12(4):811–815.
[23] Fahy JV, Corry DB, Boushey HA. Airway inflammation and remodeling in asthma. Curr Opin Pulm Med, 2000, 6(1):15–20.
[24] Fahy VJ. Remodeling of the airway epithelium in asthma. Am J Respir Crit Care Med, 2001, 164(10 Pt 2):S46–S51.
[25] Kalluri R, Weinberg RA. The basics of epithelial–mesenchymal transition. J Clin Invest, 2009, 119(6):1420–1428.
[26] Salvato G. Some histological changes in chronic bronchitis and asthma. Thorax, 1968, 23(2):168–172.
[27] Bourdin A, Neveu D, Vachier I, Paganin F, Godard P, Chanez P. Specificity of basement membrane thickening in severe asthma. J Allergy Clin Immunol, 2007, 119(6):1367–1374.
[28] Saglani S, Molyneux C, Gong H, Rogers A, Malmström K, Pelkonen A, Mäkelä M, Adelroth E, Bush A, Payne DN, Jeffery PK. Ultrastructure of the reticular basement membrane in asthmatic adults, children and infants. Eur Respir J, 2006, 28(3):505–512.
[29] Woodruff PG, Fahy JV. Airway remodeling in asthma. Semin Respir Crit Care Med, 2002, 23(4):361–367.
[30] Bergeron C, Tulic MK, Hamid Q. Tools used to measure airway remodelling in research. Eur Respir J, 2007, 29(3):596–604.
[31] Ferrando RE, Nyengaard JR, Hays SR, Fahy JV, Woodruff PG. Applying stereology to measure thickness of the basement membrane zone in bronchial biopsy specimens. J Allergy Clin Immunol, 2003, 112(6):1243–1245.
[32] Wilson JW, Li X. The measurement of reticular basement membrane and submucosal collagen in the asthmatic airway. Clin Exp Allergy, 1997, 27(4):363–371.
[33] Chetta A, Foresi A, Del Donno M, Bertorelli G, Pesci A, Olivieri D. Airways remodeling is a distinctive feature of asthma and is related to severity of disease. Chest, 1997, 111(4):852–857.
[34] Kim ES, Kim SH, Kim KW, Park JW, Kim YS, Sohn MH, Kim KE. Basement membrane thickening and clinical features of children with asthma. Allergy, 2007, 62(6):635–640.

Corresponding author
Irina-Draga Căruntu, Professor, MD, PhD, Department of Morphofunctional Sciences – Histology, "Grigore T. Popa" University of Medicine and Pharmacy, 16 University Street, 700115 Iassy, Romania; Phone +40727–003 700, e-mail: irinadragacaruntu@gmail.com

Received: November 3, 2015

Accepted: April 8, 2016