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Apoptosis quantification at the respiratory epithelium level in asthma

Research Article

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Abstract: The „changes" occurring in the expression of the factors involved in the apoptotic chain at the level of the respiratory epithelium in asthma is still an unsolved issue. At this level an important role is played by the mitochondria and the factors that influence the membrane permeability, especially the Bcl-2 super family. The purpose of this study is to evaluate both the changes in the expression of the Bcl-2 and of the Bax proapoptotic factors at the respiratory epithelium level in 21 patients with bronchial asthma of different degrees of severity of disease (according to GINA – Global Initiative For Asthma). To accomplish this, fragments of the bronchial mucosa were obtained through fiberbronchoscopy, being afterwords hystologically prepare in view of the immunomarking with anti Bcl-2 and anti-Bax antibodies. Microscopic examination revealed an important decrease in the level of proapoptotic factor Bcl-2 in patients with persistent severe forms of the disease and a significant decrease in the expression of the proapototic Bax factor at the respiratory epithelium level even in the early stages of the disease. Knowing all the factors involved in apoptosis at the respiratory epithelium level in bronchial asthma, as well as of their expression changes will be at the core of new therapeutical approaches to of this disease.

Keywords: *Bronchial asthma • Apoptosis • Bax • Bcl-2 • Respiratory epithelium*

1. Introduction

Asthma involves a chronic aggression exerted over the respiratory epithelium. The epithelium becomes fragile, which is morphologically expressed through aspects of total or partial denudation. The respiratory epithelium undergoing hyperplasic transformation of the goblet cells and sometimes lesions of squamous metaplasia that represents at the same time an important center of cytokines secretion. The epithelial response is manifested by an increase in the cell proliferation rate and by ,,changes" of the cell surviving rate secondary to the intervention of the proteins involved in regulating apoptosis. The increase in epithelial cell apoptosis observed in asthma patients is in part the consequence of cytotoxic factors produced by inflammatory cells. But recent research suggests that apart from these, corticosteroid treatments (including inhalatory

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treatments), one which is commonly used in this disease, might interfere [1]. As a consequence, more recent studies want to understand the mechanism and the factors involved in the apoptotic chain at the level of the respiratory epithelium in asthma, these results that will definitely have consequences on the therapeutic approach in asthma.

Of molecules involved in regulating apoptosis most widely studied in asthma is antiapoptotic factor Bcl-2. It participates in regulation of mitochondrial membrane permeability in balance with other molecules proapoptotic role of Bcl-2 family such as Bax and Bak.

The main objective of this study is to observe the presence and the quality of expression of antiapoptotic Bcl-2 factor and Bax proapoptotic factor at the respiratory epithelium level in patients with bronchial asthma with different degrees of severity.

2. Material and Methods

We considered a number of 21 asthma patients that were examined in the Outpatient Section of the Respiratory Disease Clinic from Iasi between Jan 2007-Aug 2008.
Inclusion criteria were:

- Non-smoking patients (actually non-smokers)
- A diagnose of asthma before undergoing fiberbronchoscopy (relevant history, bronchial obstruction with significant reversibility to inhaled bronchodilators)
- Patient without an acute or chronic disease other than asthma
- Partially controlled or totally controlled asthma at the moment of examination, according to GINA 2006 (control questionnaire of asthma - Asthma Control Test - ACT ≥ 20)
- All patients are under anti-inflammatory treatment (inhaled corticosteroid)
- Patient ability to correctly perform a spirometry that can cover acceptability and reproductibility criteria
- Patient agreement to undergo fiberbronchoscopic examination with bronchial biopsy.
- Exclusion criteria were:
- Smoking patients
- Patient disapproving of a fiberbronchoscopic exam
- Relative contraindications of performing a fiberbronchoscopy (age over 75 yo, hemathological diseases, advanced kidney or liver failure, severe obstruction – FEV1< 25% (Forced Expiratory Volume in 1 Second) of the predicted or under 1 l/sec, terminally ill patients, pathologically conditions that prohibit xilin administration, major heart diseases as recent myocardial infarction or severe rythm disturbances, other severe diseases).

Patients were evaluated through history and spirometry, and disease was diagnosed according to GINA 2002 severity scale.

At a later stage we performed fiberbronchoscopy with biopsy of the bronchial mucosa from the medium or lower lobe bronchia. Fragments were preserved in formol and included in paraphine, being afterwards colored through the standard hematoxiline-eozine method, in order to evaluate the quality of biopsies so that they uniformly respond to generally accepted criteria:

- Fixation and coloring
- Zone of the conjunctiva under the epithelium of minimum 0,3 mm for the obtained fragments
- Laceration of the tissue structure due to prelevation methods
- Without hyaline cartilage, mucus and extended hemorrhage areas.

In order to avoid inconclusive or insufficient products, we have obtained 5-7 different tissue fragments from each patient.

To achieve the main objective of our study, the fragments of bronchial mucosa were exposed to immunoassaying techniques with anti-Bcl-2 antibodies (mouse anti-human Bcl-2 oncoprotein, code N 1587) and anti-Bax (Daco, code A3533), through avidine-biotine-peroxidase method. The antibody-antigen reaction was objectified through a cromogen basis 3,3-diaminobenzidine (DAB). Evaluation of results was made through a semi quantitative method according to the immunoassaying performed on fragments of normal bronchial mucosa (2 witnesses).

Microscopic exam was performed with Eclipse E600 Nikon microscope and images with DN100 camera, Lucia Net.

For statistical analysis of results we used specific tests. For example, in the parameter analysis, Newman-Keuls test was applied, which is a non-parametric test, using a more complex method to compare data (the critical rank analysis). This is especially used in testing the difference of average values of two sets of values, in the case where ANOVA cannot be applied.

In the case of testing the level of association (correlation) we have used specific tests of correlation for quantitative and qualitative variables, such as Pearson, CHI-square (χ^2), Spearman, Gamma.

After applying these tests we have taken into discussion important parameters and according to their results we have established these conclusions. Therefore, p- the reference parameter calculated during tests, represents the significance level of the test, compared to $p= 0,05$, corresponding to a confidence level of 95%, this having significant values for calculated p <0,05.

Table 1. General characteristics and functional parameters of the patients included in our study.

Patients with bronchial asthma (n = 21)	Average values ±DS / n	min	Q25	Average	Q75	max	P(95%CI)	
Average age (yo)	44,1 ±16,1	22	27	42	56	70		
Intermitent (n=4)	26±	22				28		
Mild asthma (n=6)	38,16±	24				65		
Moderate (n=8)	57,3 ±9,8	40	52	56,5	65,5	70	0.01174	ss**
Severe (n=3)	45 ±5,2	42	42	42	51	51	0.12933	ns*
Male/Female	10/11							
Social criteria (urban/rural)	17/4							
Average lenghth of asthma (years)	16,7 ± 9,8	1.0	12.0	20.0	23.0	30.0	0.26792	ns
FEV1 (L/sec)	3.1±0.8	2.1	2.5	3.0	3.7	4.5		
Intermitent (n=4)	3.6±0.7	2.9	3.0	3.6	4.2	4.2	0.023698	ss
Mild asthma (n=6)	3.6±1	2.1	3.0	3.7	4.4	4.5	0.035882	ss
Moderate asthma (n=8)	2.7±0.6	2.1	2.2	2.5	3.0	3.7	0.047305	ss
Severe asthma (n=3)	2.9±0.7	2.5	2.5	2.5	3.7	3.7	0.021146	ss
FEV1 (% from predicted)	79.3%±20.3%	45.0%	62.0%	79.8%	95.0%	116.0%		
Intermitent (n=4)	98.0%±12.4%	88.0%	90.4%	93.9%	105.5%	116.0%	0.000179	ss
Mild asthma (n=6)	97.8%±4.5%	92.1%	93.5%	98.2%	102.0%	103.0%	0.000178	ss
Moderate asthma (n=8)	67.4%±7.0%	61.0%	62.0%	64.5%	72.6%	79.8%	0.000159	ss
Severe asthma (n=3)	49.2%±4.4%	45.0%	45.0%	48.8%	53.7%	53.7%	0.000161	ss
BPI (%)	71.4%±10.1%	55.8%	63.0%	73.5%	77.1%	91.5%		
Intermitent (n=4)	81.2%±5.9%	76.7%	76.9%	79.3%	85.5%	89.4%	0.013995	ss
Mild asthma (n=6)	77.1%±8.8%	64.6%	73.5%	76.6%	79.9%	91.5%	0.009717	ss
Moderate asthma (n=8)	65.3%±7.7%	55.8%	59.5%	62.7%	73.4%	75.1%	0.029391	ss
Severe asthma (n=3)	63.0%±5.2%	57.6%	57.6%	63.4%	68.0%	68.0%	0.029166	ss

** ns – statistically insignificant for p>0.05 (95%CI)*
***ss – statistically significant for p<0.05 (95%CI)*

3. Results

After clinical examination and pulmonary function tests were performed, the patients from the studied group were divided in the following groups of diseases severity acording GINA: 4 cases of intermitent asthma (stage 1), 6 cases of persistent mild asthma (stage 2), 8 cases of persistent moderate asthma (stage 3), 3 cases of persistent severe asthma (stage 4).

Clinical data and function parameters of the examined patients are presented in Table 1.

Following microscopic examination we have detected a greater expression of antiapoptotic factor Bcl-2 at the level of respiratory epithelium in patients with milder forms of asthma (stages 1 and 2) (Figure 1 – A) and a clear tendency to decrese the expression in patients with severe persistant forms (Figure 1 - B). Looking at the data presented in table 2, we have depicted an important expression of Bcl-2 (+++) at the bronchial epithelium level in all 4 patients with intermitent asthma (100 %), in 3 patients with mild persistent asthma (50%), in 1 patient with moderate persistent asthma and in 2 cases (66,67 %) with severe persistent asthma. The last finding was not objectified in any patient with intermitent and mild persistent symptoms.

We also found out that Bcl-2 positive cells were mainly disposed in the neighbourhood of the basal membrane in all asthma patients.

An exception to this rule were the epithelial teritories that presented squamous metaplazic lesions (independent of the degree of severity of bronchial asthma), where an important number of Bcl-2 positive cells was noticed, which extended to superficial layers (Figure 1 - C).

While examining fragments of bronchial mucosa taken from the 2 witnesses we have found a lack of Bcl-2 expression (negative reaction) at the respiratory epithelium level.

Statistic analysis performed in order to establish distribution of cases according to Bcl-2 level compared to severity degree of asthma, expressed through FeV1 levels, revealed the existence of a significant reverse corrrelation between the degree of asthma and the expression of antiapoptotic Bcl-2 factor, an important expression of Bcl-2 being found in intermittent asthma (Figure 2).

Figure 1. A. Intense positive reaction (+++) at the level of bronchial epithelium (basal cells area) – intermittent asthma, IHC with anti-Bcl-2 antibodies x 600 B. Slightly positive reaction (+) at the bronchial epithelium level – persistent severe asthma, IHC with anti-Bcl-2 antibodies x 600 C. Moderately positive reaction (++) at the level of the bronchial epithelium (area of squamous metaplazia) – severe persistent bronchial asthma, IHC with anti-Bcl-2 antibodies x 600 D. Witness from the bronchial mucosa – positive reaction (+++) at the respiratory epithelium level, IHC with anti-Bax antibodies X 600 E. Slightly positive reaction (+) at the respiratory epithelium level – mild persistent asthma, IHC with anti-Bax antibodies x 600 F. Slightly positive reaction (+) at the respiratory epithelium level (in an area with epithelium distruction) – severe persistent asthma, IHC with anti-Bax antibodies x 600.

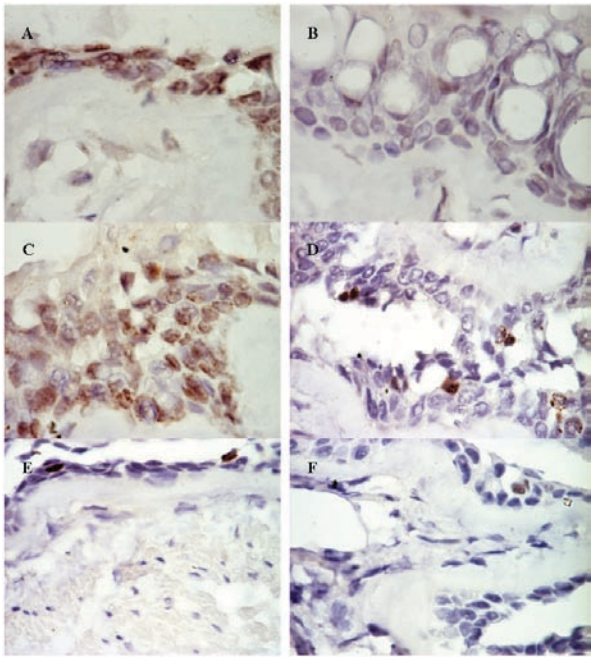


Table 2. Case distribution according to Bcl-2 vs degree of asthma severity.

Bcl-2	Intermittent	Mild persistent	Moderate persistent	Severe persistent
Mild (+)	0	0	3	2
%	0.00%	0.00%	37.50%	66.67%
Moderate (++)	0	3	4	1
%	0.00%	50.00%	50.00%	33.33%
Important (+++)	4	3	1	0
%	100.00%	50.00%	12.50%	0.00%
Total	4	6	8	3

This distribution was statistically significant: M-LChi-square=17.50755, p≤ 0.00759 (Table 3). We have also found a direct correlation statistically significant between the level of Bcl-2 expression and the severity degree

Figure 2. Case distribution according to Bcl-2 vs. degree of asthma severity.

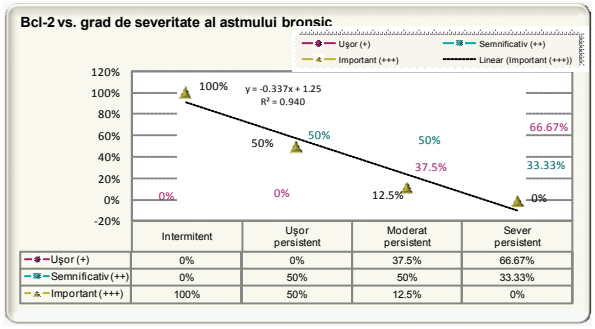


Table 3. Estimated values in testing the association of Bcl-2 vs. severity degree of bronchial asthma.

	Chi-pătrat χ^2	df	p 95% confidence interval
Pearson Chi-pătrat - χ^2	14.15312	df=6	0.02798
M-LChi-square	17.50755	df=6	0.00759
Correlation test (Spearman Rank R)	0.7442581		0.00011

Table 4. The result of correlation test of Bcl-2 level vs. severity degree of bronchial asthma .

Level Bcl-2 vs. severity degree of bronchial asthma	r-correlation coefficient (95% confidence interval)	p
Gamma Test	-0.7349	p<0.001

Table 5. The result of correlation test of Bcl-2 vs. FEV1.

Level of Bcl-2 vs. FEV1(%)	r-correlation coefficient (95% confidence interval)	p
Gamma Test	0.79734	p<0.001

of asthma (r=0.73, p<<0.01) as depicted in the data presented in Table 4.

Statistics revealed that FEV1 (%) values present a direct correlation with Bcl-2 level (r=0.79, p<<0.01), this being demonstrated by the high value of correlation coeficient and the very low value of the test significance (Table 5 and Figure 3 - A).

The same statistically significant direct correlation was noted between the level of expression of Bcl-2 and the average value of FEV1 (% from predicted) applied to severity degree of disease (Figure 3 - B).

Examining tissue fragments immunomarked with anti-Bax antibodies revealed an important expression of this factor at the repiratory epithelium level in the 2 witnesses (Figure 1 - D) and a significant reduction of Bax positive cells in asthma patients (Table 6). There was no „gradual" decrease of the Bax expressing cells in asthma patients at the epithelium level coresponding to

Figure 3. Statistical analysis Bcl-2 vs FEV1. A) Regression curb corellation of Bcl-2 vs FEV1 (%). B) Average value of FEV1 (%) according to the Bcl-2.

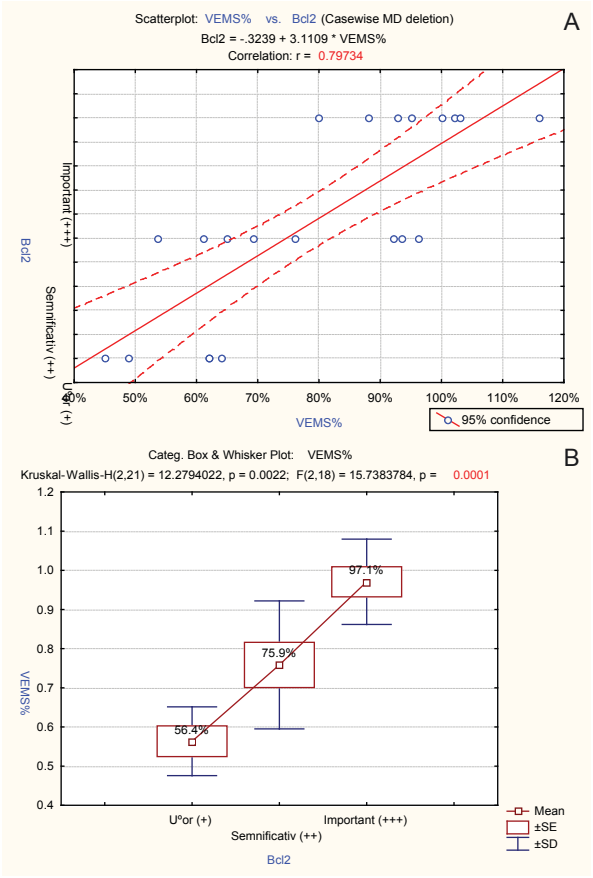


Table 6. Case distribution according to Bax vs. severity degree of bronchial asthma.

Bax	Intermittent	Mild persistent	Moderate persistent	Severe persistent
Mild (+)	4	5	7	3
%	100.00%	83.33%	87.50%	100.00%
Significant (++)	0	1	1	0
%	0.00%	16.67%	12.50%	0.00%
Total	4	6	8	3

the severity of asthma expressed through FEV1 results, few Bax-positive cells being found in intermittent and severe forms of asthma (Figure 4). Bax positive cells showed a variable position in the width of the respiratory epithelium in intermittent and mild persistent asthma (Figure 1 - E). The same aspect of „positive isolated cells" was maintained also in more severe forms of asthma (moderate and severe persistent), independent of the nature of epithelial changes such as: goblet cells hyperplasia, sqamous metaplasia or epithlial desquamation (Figure 1 - F).

Figure 4. Case distribution according to Bax level vs. severity degree of bronchial asthma.

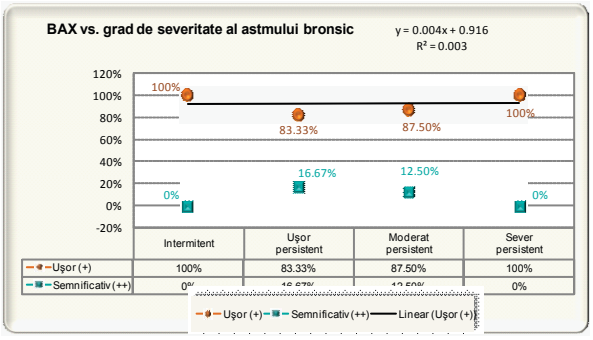


Table 7. Estimated parameters in testing of Bax association vs. severity degree of bronchial asthma (study group).

	Chi-pătrat χ^2	df	p 95% confidence level
Pearson Chi-square - χ^2	1.174342	df=3	0.75916
Yates Chi-square	1.773615	df=3	0.62069
Correlation coefficient (Spearman Rank R)	0.00807		0.972

The results of nonparametric tests, applied in view of testing the degree of association of proapoptotic Bax factor at the bronchial epithelium level, with the degree of asthma severity, does not indicate a significant association between these (χ^2=1.77, p=0.6206) (Table 7). This aspect can be explained by the moderate presence of Bax positive cells in the slight and moderate forms of bronchial asthma and the extremely rare presence of these cells in the severe forms.

The same observations, namely the absence of a directly significant correlation between the degree of the severity of disease and the Bax expression level (r=0.00807, p=0.972, 95%CI) can be observed from the analysis of regression curb of the Bax level vs. degree of disease severity (Figure 5 - A).

We have also noticed that FEV1 values present a direct and significant correlation with the presence of Bax positive cells (r=0.173, p=0.452, 95%CI) (Table 8 and Figure 5 - B).

At the same time, average values of FEV1 (%) don't express significant differences in relation with the Bax positive cells (p=0.451, 95%CI) (Figure 6).

4. Discussion

The apoptosis of the bronchial epithelium cells is up to point a physiological mechanism that assures keeping the regular number of cells, the regeneration of the epithelium in case it is subject to aggressions and

Figure 5. Statistical analysis Bax vs FEV1. A) Regression line in the correlation between Bax vs. asthma severity degree. B) Regression line in the correlation of Bax vs. FEV1 in bronchial asthma.

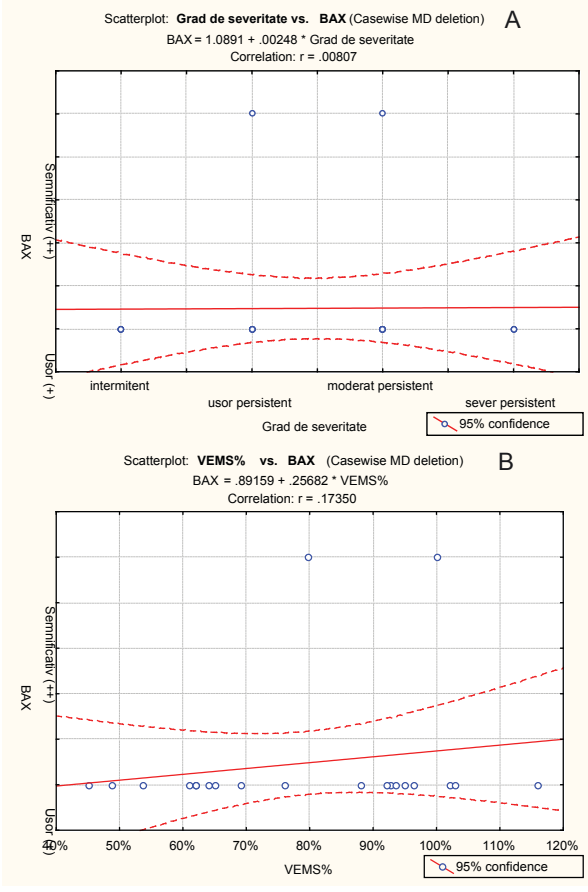


Table 8. The result of correlation test of the level of Bax vs. degree of severity of bronchial asthma.

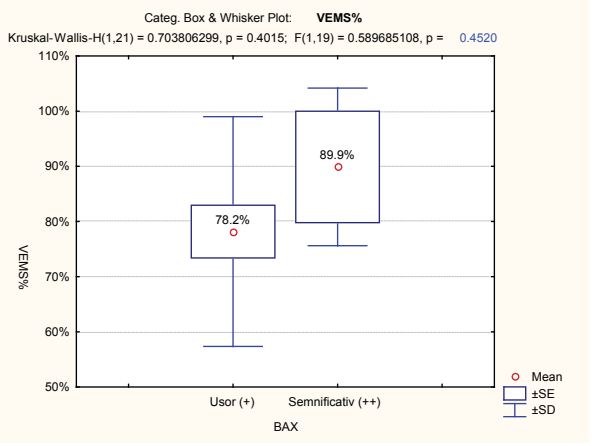
Bax level vs. FEV1 (study group)	r-correlation coefficient (95% confidence level)	p
Spearman Rank-R test	0.1735	0.452

prevents the expanding of a local viral infection.

The idea of regeneration of the bronchial epithelium through its two mechanisms, proliferation and prolongation of the cellular life by a perturbation of the apoptosis mechanism of the epithelial cells it is still, after almost twenty years, modern because data regarding the degree of expression of factors that regulate apoptosis in the asthmatic disease, are not very clear. It is even more difficult, because only a few aspects about the regular apoptosis of the bronchial epithelium are known concerning mainly the ciliary cells, and about the goblet cells very little is known while about the regeneration cells nothing [2].

Most of the researches done so far in this respect are emphasized on the degree of expression of

Figure 6. Average value of FEV1(%) vs. Bax level.



the antiapoptotic factor Bcl–2 from the level of the bronchial mucosa in the asthmatic disease. The human protooncogene Bcl–2 is located at the level of the 18th chromosome, and its encoded proteins are located at the level of smooth endoplasmic reticulum membranes, the nuclear membrane and the external mitochondrial membrane with the aim of preventing the activation of the caspase system, therefore blocking the apoptosis of the cells.

The studies done so far led to contradictory results because of the variability of the disease but also due to the technical conditions of evaluating the results of the immunohistochemical exam with Bcl–2 antibodies.

There are researches as Vignola`s and collaborators done on 9 asthmatic untreated patients, 9 asthmatic patients under corticosteroid inhaler treatment and 19 asthmatic patients addicted to cortisone, to which the authors observed an increase of the antiapoptic factor Bcl–2 in comparison with the witnesses (16 people) [3].

The authors of a new research done on 27 people (9 witnesses, 9 asthmatic untreated patients and 9 asthmatic patients under corticosteroid treatment) came to the same conclusions. After the immune marking with Bcl–2 of the fragments of bronchial mucosa gathered through fiberoptic bronchoscopy, the authors noticed and increase of Bcl–2 factor at the level of external epithelium (especially the basic layer) at the asthmatic patients in comparison with the witnesses. Furthermore, the number of the Bcl- 2 positive cells was correlated to the intensity of expression of the proliferation factor PCNA. The authors of the same research noticed the presence of a greater number of positive cells Bcl–2 in the structure of the bronchial epithelium to the people under corticosteroid treatment in comparison with those without there is a likelihood that the steroid treatment could make proliferation easier and the cellular survival while the Bcl- 2 could be a marker of this phenomenon [4].

The same observations, that is an increase of expression of the Bcl–2 factor at the level of epithelial cells is mentioned, by other researchers as well, who consider that this fact shows the existence of a self defense ability of the bronchial epithelium from the aggression of the inflammatory stimuli [5].

Recent researches shown a decrease of Bcl-2 expression at the bronchial epithelium level in asthma. In this respect, the noticeable results published by Cohen & collaborators in a research done on 9 patients with persistent mild asthma and 31 patients with persistent severe asthma and 21 witnesses shows a decrease of the antiapoptotic factor Bcl-2 at the level of the bronchial epithelium to asthmatic patients in comparison with regular people [6].

The decrease of the Bcl-2 expression level, on our study, shows an increase of cellular survival at the level of bronchial epithelium, this fact and the increase of expression of the cellular proliferation factors (Ki67) defend the thesis of the existence of an "repair phenotype" the epithelium in asthma.

Issue of the degree of expression of Bcl-2 antiapoptic factor in the bronchial epithelium is still on debate, the more actual the idea of prescribing inflammatory corticosteroids in asthma (especially in large quantities when complications occur) lead to "lesion" of the bronchial epithelium.

It is acknowledged and accepted by all the researchers the anti-inflammatory effect of corticosteroids. Their use leads to an increase of the inflammatory cells apoptosis (eosinophils and lymphocytes T) from the level of the bronchial mucosa, with the secondary decrease of their number and the quantity of the proinflammatory factors produced by these cells.

At the epithelia level, the corticotherapy effects proved to be variable: cutting down the after lesion regeneration of the epidermis [7], but has an antiapoptotic effect at the level of epithelial cells from the mammary glands [8,9] or the alveolar epithelium [10].

As a result, one of the modern issues of asthmatic pathology is to establish the ,,effect" of the corticosteroid treatment (influenced perhaps by the quantity and administration period) of the bronchial epithelium.

Mitochondria functions as a sensorial ,,cellular stress". This stress, that can be produced by corticosteroids, may include redox reactions, oxidizing effects generated by extrinsic stimuli or the variation of the cytokines production. Mitochondria may work as a ,,carrying out center of apoptosis" through different signals in a final stage of the beginning of apoptosis.

In this respect, there is research [11] showing the treatment with dexametazone leads to the depolarization of the mitochondrial membrane releasing C cytochrome and activating caspaze 9. The proapototic mode is controlled by proapoptotic molecules and antiapoptotic molecules from Bcl-2 family.

According to other researches glucocorticoids in small doses are useful in reducing the inflammatory process, and the lack of inflammation would regenerate the bronchial epithelium. Large doses of steroids could lead to an increase of epithelial lesions, apart from the inflammation of the bronchial mucosa [12]. Therefore, the idea of keeping corticosteroids as a basic therapy in asthma is encouraged nowadays, along with the prescription of drugs (β-adrenergic agonists and leukotrienes antagonist) in asthma and not increasing the dose of anti inflammatory steroids.

It is known that glucocorticoids lead to the apoptosis of the hematopoietic cells in 24 hours and in a large amounts (>75% of the cells) [11]. Although the spreading of the apoptotic effect is substantially smaller (about 10% of the cells) in the bronchial epithelium, the chronic prescription effect on cells with a low proliferation index might become important in the future [11].

The results of the researchers of asthma attest the apoptosis growing effect of the bronchial epithelium cells (in vitro) under the influence of corticotherapy. Furthermore, this effect was observed at similar quantities to those used in everyday practice. The examination of this phenomenon is done by the apoptotic regulatory molecules (as Bcl-2, Bax). As a result, it is possible that soon, the hypothesis that glucocorticoids represent one of the ,,lesion" factors and remodelation factors of the bronchial epithelium in asthma comes true.

The emergence of a great number of goblet cells in the bronchial epithelium it is not a characteristic of asthma, this process being in fact an ,,answer" of the epithelium towards the aggressions of polluting substances, irritating particles and microorganisms [13,14].

The mucus hypersecretion of the asthmatic patients has two origins: the goblet cells of the epithelium and the glandular mucous cells. Hyperplasia and the degranulation of goblet cells mechanisms are not fully cleared up nowadays. It is known that LyT4 and the cytokines produced by these cells are important factors that determine the increase of the number of the goblet cells in asthmatics [15].

Modern research in the physiopathology of the hypersecretion of mucus from the bronchial epithelium level, as well as on the goblet cells hyperplasia mechanism, focus on the role of several molecules involved both in the regulation of mucin exocytosis (such as MARKS - myristoylated alanine-rich C kinase) and also in the increase of the number of the goblet cells (such as EGF - Epidermal Growth Factor and CLCA -

calcium activated chloride channels) or in keeping a large number of goblet cells (Bcl-2 and Bax factors) [13].

Despite all the effort made so far, the hypersecretion of mucus is still an important indicator of morbidity and mortality in the pulmonary pathology (bronchial asthma included). Therefore, the issue of the mucus hypersecretion cure in the pulmonary pathology is still on debate, the lates information concerning the likelihood of controlling the mucus quantity of the breathing apparatus is focused on the holding back of the nervous activity (with the help of antagonists of tahikinine receptors or inductors of epoxygenose) or the holding back of mucin exocytosis (anti-MARKS, blocking Munc-18B), synthesis and of goblet cells hyperplasia (with the help of EGF inhibitors and tirozinkinase receptors and mitogen–activated protein p38) and last but not least leading to the apoptosis of the caliciform cells (Bax factor or Bcl-2 inhibitors) [16].

There are only a few scientific studies on the role of Bax proapoptotic factor in the apoptosis mechanism from the level of bronchial epithelium in asthma, due to the fact that it is a new issue and also complicated to gather bronchial mucosa as the fiberoptic bronchoscopy is not regularly done to asthmatic patients.

In this study one can notice a decrease of the Bax proapoptotic factor from the level of bronchial epithelium even to the patients with intermittent forms of asthma in comparison with the witnesses.

Schwalm K. and his collaborators in a recent study, realized on fragments of bronchial mucosa taken after the death of patients with both mild and severe forms of asthma, noticed a decrease of Bax expression at the level of goblet cells of the bronchial epithelium, in comparison with the witnesses [17]. According to the results, the authors of the research noticed a decrease of Bax expression to the asthmatic patients, even from the precocious disease stages, supporting the hypothesis that Bax proapoptotic factor could reestablish the regular number of cells from the bronchial epithelium level and, furthermore reduce the mucus hypersecretion leading to the apoptosis of the goblet cells [17].

The observations of another research support these results, a decrease of the number of goblet cells from lab animals under the influence of interferon γ, which determines the activation of Bax proapoptotic factor [18].

Experimental researches proved that IL-9 and IL-13 lead to goblet cells metaplasia at the bronchial epithelium level. This process is reduced by interferon cure, an activating factor of apoptosis of these cells by means of a Bax mechanism [19].

Regularly, Bax is usually located in citosol, but after the apoptotic signals he undergoes a series of conformational changes and can be found at the membrane level of different cellular organites (especially at the level of extrinsic mitochondrial membrane) [20,21]. At his level, Bax could favour the opening of the voltage-dependent mitochondrial channels (VDAC) [22] and permit the permeability of the mitochondrial membrane, releasing the C cytochrome in the cytoplasm and other proapoptotic factors (Smac/DIABLO, Omi), that would activate the caspase [23].

Most of the researchers agree with the idea of mitochondrial permeability pores formed from voltage–dependent channels (VDAC or porine) at the extrinsic mitochondrial membrane level and another protein from the intrinsic mitochondrial membrane level (translocator adenin nucleotidic or ANT). The direct interaction of the two mitochondrial proteins would have as a result a new VDAC – ANT complex that could lead to the approaching of the two mitochondrial membranes with a permeability pores at mitochondrial level [24,25].

The role of antiapoptotic factors from the BCl-2 family (such as BCl-2 protein) is at the mitochondrial membrane level as well „blocking" the release of C cytochrome [25].

The issue of mucus hypersecretion and the perturbation of the apoptotic phenomenon from the bronchial epithelium level, is still open, future research will go deeper into the subject of these phenomena.

The decrease of expression of Bax proapoptotic factor from the bronchial epithelium level is an element that helps rendering goblet cells` life longer and maintains a high level of mucus secretion in asthma.

These observations are comparable with the results of other study from the literature, with the emphasis that the posibility of a therapeutic „control" of the expression of these regulatory factors of cell apoptosis will open new way for a therapeutical approach in asthma.

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