"OVIDIUS" UNIVERSITY OF CONSTANȚA
DOCTORAL SCHOOL OF THE FACULTY OF MEDICINE

Ph.D. FIELD- MEDICINE

DOCTORAL THESIS ABSTRACT

Bronchial hyperreactivity in obstructive respiratory pathology in children

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TABLE OF CONTENTS

Keywords: bronchial hyperreactivity, wheezing, child

INTRODUCTION

During the last three decades it has been given a great importance to the non-invasive explorations of bronchial inflammation emphasizing the level of pathophysiological substrate in the diagnosis, treatment and outcome of patients with obstructive pulmonary disease.

From an etiopathgenetic point of view, it is more and more believed that the increased bronchial hyperreactivity is the common functional element of the nonspecific obstructive pulmonary diseases.

Bronchial hyperreactivity (BHR), defined as excessive bronchial constriction, occurs as an exaggerated bronchoconstriction response of the airways that occurs as a cause of nonspecific stimuli.

The main objective of this dissertation is to evaluate the eosinophilic inflammation in children with bronchial hyperreactivity and to identify the factors that trigger asthma. This is why I used the dosage of the eosinophil cationic protein (ECP) in serum as a marker of the eosinophilic inflammation, which can predict the persistence of wheezing in children older than 5 and therefore the diagnosis of asthma. In Romania, it is only the beginning of the research in highlighting the role of ECP, which can be measured by using different body fluids such as serum, plasma, sputum, saliva, fluid obtained by nasal and bronchial-alveolar lavage.

By comparing all possible methods to analyze ECP, I chose the serum as a fluid because the ECP level in it is 5-10 times higher than in other fluids. [1]. The determination of ECP in sputum was excluded as a method of analysis, because my study included young children (2 to 4 years of age), noncompliant in performing the necessary collection maneuvers. The dosage of ECP in the fluids obtained from nasal and Broncho alveolar lavage was also ruled out since it is an invasive method, which was refused by parents in most cases.

Thus, the main objective of this dissertation is the evaluation of the use of ECP dosage in serum as a marker of inflammation in children with asthma.

I. BRONCHIAL HYPERREACTIVITY DEFINITION AND PREVALENCE

BHR is defined as an excessive bronchial constriction which appears as an exaggerated bronchoconstriction response of the airways that occurs as a cause of nonspecific stimuli.[2]

The main feature of the BHR is the inadequate response given by smooth muscle of the airway after direct or indirect exposure to a variety of exogenous stimuli-determined or

endogenic-predisposing (allergens, chemical or pharmacological substances, cold air, viral infections hypoxia etc.).[3].

BHR prevalence is estimated to be between 10% and 25% [4,5,6], way above the rate of asthma (just over 5% of the general population). [7]

Although bronchial hyperreactivity often acquired in early childhood is a pathognomonic feature of asthma, that it is not entirely specific to this and it can also be found in a wide range of respiratory diseases such as chronic rhinitis, upper respiratory tract infections or may occur in patients with normal respiratory function. [8]

II. MAIN MECHANISMS INVOLVED IN BHR OCCURANCE

BHR occurrence (the syndrome connected to asthma) involves the following: mast cells, lymphocytes, macrophages, eosinophils, bronchial epithelial cells, lymphocytes, monocytes, basophils, polymorphonuclear cells and other cells. The route of the cells to the site of inflammation is monitored by chemical signals and is based on three steps:

A. The adhesion of the cell to the vascular endothelium- a process done by glycoproteins of adhesion , which are present on the surface of endothelial and inflammatory cells at that time.

B. The migration into tissues from where input signals are coming (in the case of eosinophil, the signal can be represented by platelet-activating factor, etc.)

C. The tissue level activation of the inflammatory cells - process achieved by interleukins (IL-5 for eosinophilic). IL-5 is a major component of the pathogenesis of asthma, but its signs are increases by macrophage products (PAF, GM-CSF = granulocyte macrophage colony stimulating factor). [9]

III. CHARACTERISTICS OF THE PEDIATRIC RESPIRATORY SYSTEM

The abdominal breathing is at a rate of 60 breaths / minute. The respiratory rate may sometimes be irregular with bouts of apnea, Cheyne-Stokes rhythm, the amplitude being uneven sometimes. The volume of air is 10 to 15 cm3. The respiratory minute volume is 600-750 cm3.

During the first 4 months, the ventilation is performed exclusively by nasal breathing which can cause respiratory difficulties when the nasal cavities are obstructed. Due to incomplete development of the chest and lungs, the pulmonary functional reserves are lower.

The obstruction of airways occurs more rapidly in children because:

- the incomplete development of elastic cartilage tissue and adjacent structures responsible for maintaining patency of these air ducts increase the risk of lower airways to collapse;

- the small diameter of the airways in children explains the high resistance to the airflow in the case of a moderate airway obstruction.[10]

Starting from the assumption that BHR is often associated with BA (at a rate of 80-100 case), I chose to focus on this condition from the group of conditions related to the BHR.

IV. ASTHMA

Asthma is a chronic inflammatory disease of the airways characterized by increased respiratory response to a variety of stimuli. [11] The pathophysiological substrate of this disease is the chronic inflammation of the airways associated with the bronchial hyper responsiveness to various stimuli.[12]

IV.1 Clinical diagnosis of Asthma

After completing the clinical examination and identifying the signs and symptoms described by patient, we can relate to the Asthma Predictive Index. This is a score that helps the doctor to predict the early risk of asthma occurrence in children under 5. Children younger than 3, who experience more than 4 episodes of wheezing per year, have an increased chance of being diagnosed with asthma after the age of 5, if they meet the following factors:

1. A major factor:

- a parent with asthma
- diagnosis of eczema (atopic dermatitis)
- sensitivity to allergens (diagnosis based on the skin or blood tests).

OR

2. Two minor criteria:

- food allergies
- eosinophilia> 4%
- wheezing not during viral episodes.13]

IV.2 Laboratory diagnosis of asthma

Depending on patient's status the following chart investigations must be completed:

- During asthma attack: blood count, erythrocyte sedimantation rate, bacteriological sampling, otorhinolaryngology exam, heart and pulmonary radiography, tuberculosis skin test (PPD skin test).
- Status asthmaticus requires other investigations, such as: pulsoximetry, blood gas analisys, blood and urinary ionogram, urinary proteins, electrocardiogram, ocular fundus examination and electroencephalogram.

- In order to establish the asthma severity are necessary : allery tests, histamine provocation test (useful in detecting airway responsiveness), different tests for excluding other conditions: cystic fibrosis screening, serum, sputum and bronchial electrophoresis, barius test, electroencephalogram, pulmonary biopsy.

a) Spirometry: In older children with an intermediate chance to develop asthma, the diagnostic tests (peak expiratory flow rate, PEFR, forced expiratory volume in one second - FEV) are the main elements in the observation of the obstruction of the airways. However, they may be normal between episodes of bronchospasm. The spirometry is usually possible after the age of 5 and it depends on the patient's cooperation and understanding of the requirements.

b) The immunological examinations (the quantitative determination of lgE) may reveal bronchial hyperreactivity seen in obstructive respiratory diseases. [14] IgE is the central mediator in the pathogenesis of allergic asthma and related diseases; most of asthmatics have high levels of IgE in the serum.[15]

c) The determination of the eosinophil cationic protein (ECP): ECP in serum is present in infants, since the first episode of wheezing, especially to those who will later develop asthma. The measurement of ECP in children helps to identify those patients who develop asthma. These findings indicate that the determination of ECP in serum can be used to identify children in need of an early anti-inflammatory treatment.[16]

d) The challenge tests with specific allergen are the only ones that establish the causal relationship between asthma and reaginic sensitization. The respiratory functional parameters are measured after the inhalation of certain progressive concentrations of allergen incriminated in the etiology of asthma.

V. SPECIAL PART

V.1. MATERIAL AND METHOD

The study was prospective, group type and it was conducted on a total of 199 patients with wheezing, which have been watched for a period of four years, between 2009 and 2013.

The study was conducted in the Pediatric Clinic of the Constanta County Emergency Hospital.

The age group of the patients analyzed during the study was 2 to 4 years and they have been complaining of recurrent episodes of wheezing.

Inclusion Criteria

Patients were included in the study if they met the following inclusion criteria:

Inclusion criteria:

- Age: 2 - 4 years old

- Presence of symptoms of bronchial obstruction (the existence of episodes of expiratory dyspnea with wheezing and cough, in the past or during the evaluation, episodes caused by a viral infection, exposure to allergens or irritants and atmospheric pollutants);

- Adherence to the study of children and families;

- Consent of parents of these children;

Exclusion criteria:

- Personal reasons of non-involvement of some children in the study;

- The presence of other chronic respiratory and cardiac diseases that can affect the test results;

- Regular administration of inhaled corticosteroids;

Children were assessed using a questionnaire to evaluate the following parameters:

-age, gender, origin;

-the age of the mother, her education, the organization of the family (marital status of the mother), socio-economic vices of parents during pregnancy / postpartum;

- previous family history

- personal physiological history (gestational age, weight at birth, completion of the immunization schedule);

-type of feeding;

- atopic environment;

- related comorbidities;

- number of episodes of bronchial obstruction prior to their introduction in the study.

QUESTIONNAIRE

1	Name and Surname			
2	Date of birth (CNP)			
3	Gender			
4	Area of origin	Rural		
		Urban		
5	Gestational age			
6	Ventilation/ Intubation at birth			
7	Weight at birth			
8	Socioeconomic conditions (atopic environment)	Mold, moisture		
		pets		
9	Atopic parents (rhinitis, sinusitis, asthma)			
10	Smoking parents			
11	Smoking during pregnancy	Active		
		Pasiv		
12	Marrital status of the mother			
13	Education of the mother			
14	Nutrition during infancy		Duration of breastfeeding	
			Hypoallergenic formulas	
			Cow's milk nutrition	
15	Completed vaccinations			
16	Associated **comorbidities**	Obesity		
		Allergic rhinitis		
		Allergic conjunctivitis		
		Atopic dermatitis		
		Food or drug allergies		
		Chronic adenoid		
17	Episodes of bronchial obstruction	Number		
		If they required hospitalization		
		If the episodes of bronchial obstruction appeared after exercise or excessive crying		

The study was conducted in accordance to the ethical principles of the "Bill of Rights" from Helsinki, taking in consideration the safety and wellbeing of patients, the supervision and the medical decisions being always in favor of the included patients.

At the first visit, the following data was recorded: severity of the disease, history of bronchiolitis, family history of asthma or atopy, associated comorbidities (atopic dermatitis). Skin tests were performed for common allergens, eosinophil cationic protein determination

(ECP), blood count from which the most relevant ones were the serum eosinophils and the total level of IgE.

The groups were evaluated every 4 years the most. I composed an evaluation plan which contained the following screening visits:

• Visit 1: completing the questionnaire mentioned above, obtaining consent from the family, evaluating the inclusion / exclusion criteria, determining the lab and allergy tests;

• Visit 2: evaluating the inclusion / exclusion criteria, determining the necessary lab and allergy tests, performing spirometry and bronchodilation tests.

At the end of the second visit, after which both groups were assessed using spirometries, it was confirmed or not the suspicion of asthma, so that 126 people were diagnosed with asthma.

The assessment of the lung function was performed to all children included in the study, after the age of 5, when they were cooperative and capable of performing the necessary maneuvers for the correct measurements. The following parameters were measured by spirometry: FVC (forced vital capacity - the amount of breath after a deep inspiration), FEV1 (forced expiratory volume in one second) and FEF 25-75 (volume of air removed from first quarter of vital capacity until the elimination of the third quarter- mid - expiratory maximum flow). We also determined the ratio FEV / FVC (Tiffen index) because it is a very sensitive parameter, the level of low (<0.7) determining the diagnosis of bronchial obstruction. This is essential for the differentiation between obstructive and restrictive diseases. The bronchodilation test was also performed to the 126 children diagnosed with asthma in order to confirm the bronchial hyperreactivity.

V.2 Group Study

The study was based on the observation of a group of patients with wheezing for a period of four years. At the end of the follow-up period, 126 cases were diagnosed with asthma.

Therefore, the patients were divided into 2 groups: one group- 126 patients with asthma (63.3%) and a 73 patients with wheezing 73 (36.7%) (Figure nr. 1)

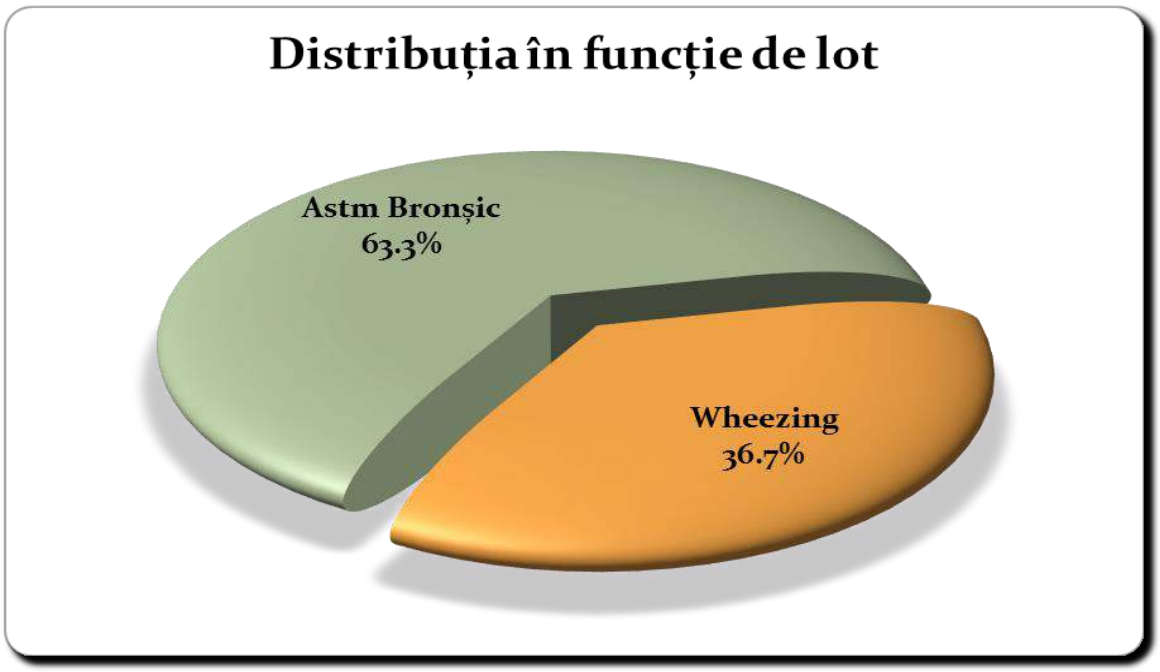

Figure 1 Clasification of patients by group study

VI. RESULTS

VI.1 Gender

The classification of the participant patients by gender shows that 63% of male patients did not develop asthma at the end of the evaluation period and 57.1% of them developed asthma. (Figure 2)

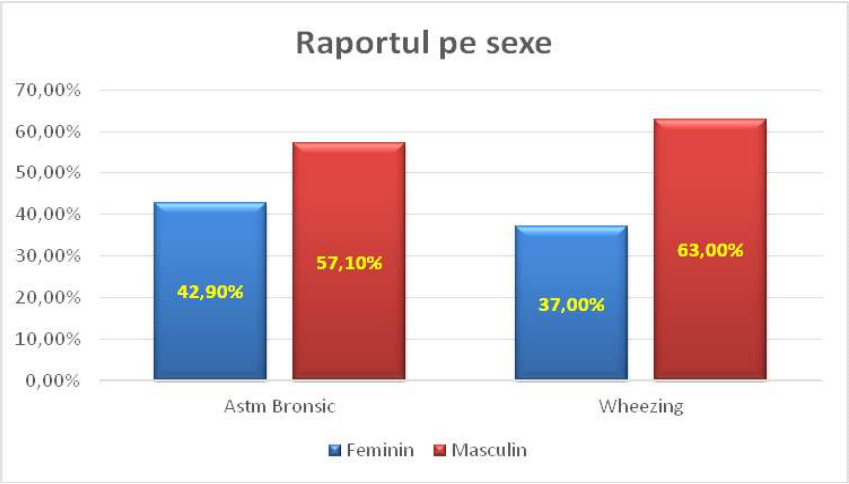

Figure 2 Classification of patients by gender

We applied the Chi-square test to determine the existence of statistically significant associations between gender and appearance of asthma. (Table I)

There is no statistically significant association between the development of asthma and the gender of the patient with a history of wheezing, $\chi 2$ (1) = 0.660, p = 0.417.

Therefore, there is no association between the gender of the patients and their possibility to develop asthma in the future.

Table I- The Chi-square test - Association between gender and occurrence of asthma

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.660a	1	.417		
Continuity Correction b	.439	1	.507		
Likelihood Ratio	.663	1	.415		
Fisher's Exact Test				.456	.254
N of Valid Cases	199				
a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 29.71. b. Computed only for a 2x2 table					

It was found that bronchial hyperreactivity in children is more common in boys than girls, as resulted from my study. When diagnosing asthma in patients with bronchial hyperreactivity by following a meta-analysis of 4 studies conducted during the period of 1989-2007, including children 0 to11 years of age, the ratio male / female was 2: 1 or greater. However, recent related studies indicated this ratio to be almost equal, but with a slight predominance of males 1: 0.9. [17] This difference is due to the fact that boys have a reduced airway caliber in comparison to their lung size, an increased muscle tone of the bronchial muscle, which causes the airflow limitation to be higher during the response to various allergens. This difference was also determined by the excess of lgE. Nevertheless, the gender distribution is reversed for teenagers, asthma occurring mostly in girls. This change is considered to be caused by hormonal differences which may influence the airway size, the degree of inflammation, the muscle tone and the vascularity of the bronchial tree. [18]

VI.2 Area of origin

By analyzing the classification of patients by area of origin it can be noticed that over 60% of the patients from urban areas developed asthma and more than 71% of children did not develop asthma. (Figure 3)

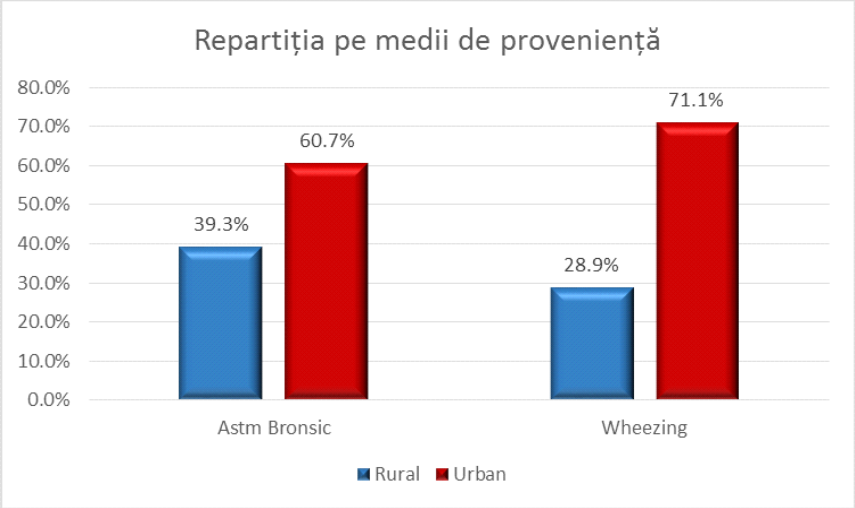

Figure 3 Classification of patients by area of origin

The objective is to determine the existence of statistically significant associations between the area of origin and the development of asthma.

The result shows that there is no statistically significant association between the development of asthma and the area of origin for children diagnosed with a history of wheezing, $\chi 2$ (1) = 1.246, p = 0.246.

Many recent records reflected an increased prevalence of asthma in densely populated areas (urban ones, developing countries) and it also highlighted the contribution of environmental factors to the pathogenesis of this disease. The concentration of allergens, their penetrance of the bronchial mucosa and even the atopic response could be affected by pollution, especially by substances such as sulfur dioxide, ozone and nitrous oxide. Sulphur dioxide can even cause a bronchoconstriction without clinical significance in healthy individuals. In patients with asthma, the clinical bronchoconstriction occurs in early stages, even at concentrations found in the urban pollutant environment. The SO2 effect is intensified by effort, when the ventilation increases.

In the urban environment of countries where the environmental protection is not a priority, high concentration pollutants would harm the respiratory epithelium, thus allowing other allergens to enter the lung. [19]

VI.3 Classification of patients by presence of atopic environment

The atopic environment is found in 31.7% of patients with asthma, and 45.2% of patients who did not develop asthma. (Figure 4).

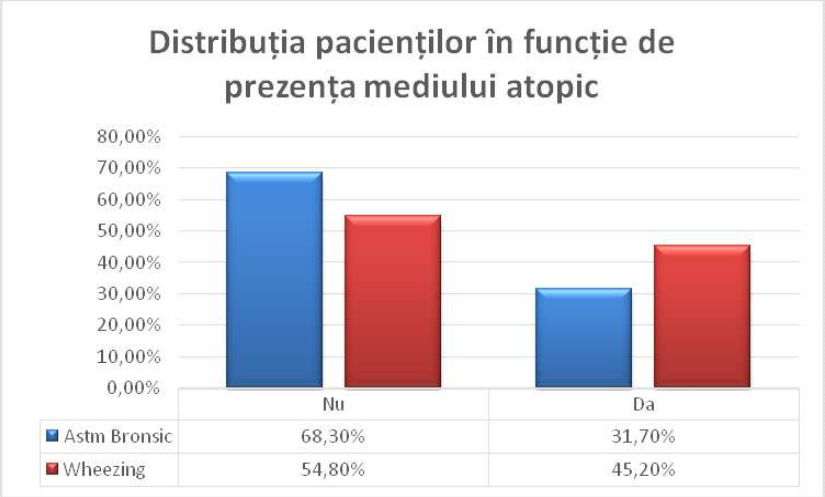

Figure 4 Classification of patients by presence of atopic environment

In order to determine the statistical significance of the differences observed between the two groups, we applied the Chi-square test. The result is not statistically significant ($p = 0.058$), therefore there is no statistically significant association between the atopic environment and the development of asthma in patients hospitalized for wheezing.

VI.4 Classification of patients by smoking during pregnancy

The percentage of patients whose mothers smoked during pregnancy has very similar levels: 50% of patients developed asthma, and 41.4% of them have not developed asthma. (Figure 5)

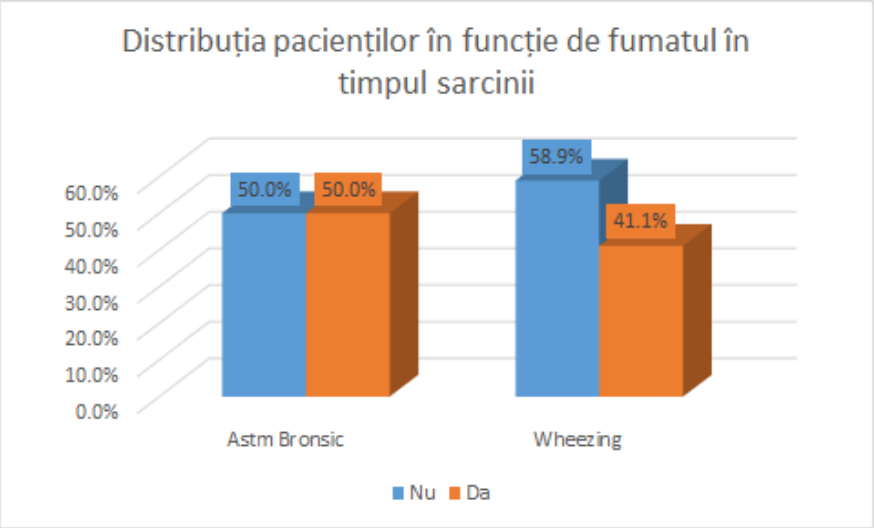

Figure 5 Classification of patiens by smoking during pregnancy

Chi-square test does not have a statistically significant result (p = 0.225), therefore there is no statistically significant association between smoking during pregnancy and the evolution of the patients in this study. (Table II). I would like to mention that in Romania the smoking during pregnancy has a percentage of 20-30%. Thus, in the studied group, represented by children with increased bronchial hyperreactivity, the percentage of the smoking population is significantly higher than the general one. [20] Also, the study conducted by Frank D. supports the idea that smoking during pregnancy was associated with significant respiratory changes in children, suggesting that fetal exposure to smoking may have significant effects on the respiratory health of the child for long-term . [21] In this study, only 161 (3% in 5762) of women quitted smoking before giving birth. [21]

Table II Chi-square test for the analysis of smoking during pregnancy

	Valoare	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.472[a]	1	.225		
Continuity Correction B	1.136	1	.286		
Likelihood Ratio	1.478	1	.224		
Fisher's Exact Test				.241	.143
N of Valid Cases	199				
a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 34,12.					
b. Computed only for a 2x2 table					

IV.5 Classification of patients by ECP levels (eosinophilic cationic protein)

The eosinophilic cationic protein was evaluated in 183 patients: 119 were subsequently diagnosed with asthma, and 64 were diagnosed with asthma at reevaluation.

For the group of patients who developed asthma, the average level of the protein eosinophilic cationic was 24.34 g / L (standard deviation of 12.14 mg / L). It is noted from the beginning that the average level of the eosinophil cationic protein is increased in comparison with the maximum reference level of the laboratory, which is 11 mg / L. For patients who have not been diagnosed with asthma, the averaged level is 17.92 mg / L, with standard deviation of 11.96 mg / L. (Table III)

It is observed even in this case that the average levels were higher than the reference one.

Table III Descriptive analysis of ECP levels

Group	N	Mean	Median	Std. Deviation	Variance	Skewness	Kurtosis
Asthma	119	24.340	21.600	12.1404	147.390	.650	-.366
Wheezing	64	17.972	13.650	11.9673	143.217	1.936	4.973
Total	183	22.113	18.600	12.4262	154.410	.980	.633

The classification of ECP levels for both conditions is presented by comparison in Figure 6. It is clear that both classifications are asymmetrical to the left, most of the levels being below 20 g / L.

By analyzing the histogram and the result of the Shapiro-Wilk test, it can be clearly observed that the distribution of ECP levels significantly differ from a normal distribution. As a result, the median is used for a more useful comparison, and the Mann-Whitney non-parametric statistical test as a comparison one.

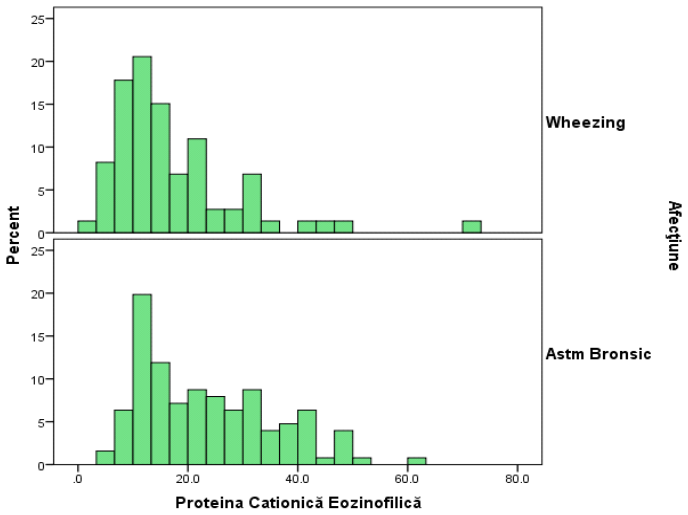

Figure 6 Distribution of ECP levels

By comparing the median level for the two groups it is observed that the level is almost 60% in patients who developed asthma, compared to the group of patients who did not develop asthma. (Table IV)

Table IV Testing the normality of ECP levels distribution

	Condition	Kolmogorov-Smirnova			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Cationic Eosinophilic Protein	Asthma	.119	126	.000	.935	126	.000
	Wheezing	.180	73	.000	.822	73	.000
a. Lilliefors Significance Correction							

The difference between the two groups is statistically significant, U = 2470.5, z = -3.914, p <0.001. (Table V)

Table V Mann Whitney ECP test

	Cationic Eosinophilic Protein
Mann-Whitney U	2470.500
Wilcoxon W	4550.500
Z	-3.914
Asymp. Sig. (2-tailed)	.000

The size effect given by the r index indicates a medium to small difference between the two groups (r = -0.289).

The applied Chi-square test indicates a statistically significant association between the ECP levels and the occurrence of asthma. (p = 0.0002).

The odds ratio of patients with elevated ECP levels who will be diagnosed with asthma is 4.37 (CI95% from 2.017 to 9.46). Therefore, a child with wheezing showing elevated ECP levels has a significantly higher risk of being diagnosed with asthma than a child with normal ECP levels.

According to a study by Ingram et al. [22], conducted on a group of children with wheezing , 2 to 4 years of age, the level of ECP in serum was similar to the one obtained in my study (24.34 mg / L). This idea is supported in another study published by J. Rvilla [23], which emphasizes that the ECP has an average level of 22.48 mg / L in children with persistent wheezing. By measuring the level of ECP, I intended to make a connection between the high levels of ECP and the clinical evolution of these children with wheezing. We have found that those subjects with higher levels of ECP (21μg / L) had a significantly increased risk of being diagnosed with asthma after the age of 5.

IV.6 Classification of patients by Immunoglobulin E levels

The average lgE level is increased in patients who were later diagnosed with asthma, averaging to 117.73 IU / ml and the median being at 95 IU / ml. The standard deviation for this group is 79.194UI / ml. For the second group, the average of these levels is 67.62, standard deviation being at 69 185 IU / ml and the median at 44.00 IU / ml. (Table VI) Therefore, it can be noticed in both cases a very large dispersion of levels, presented in the Figure 7.

Table VI Descriptive analysis of IgE levels

Condition	N	Mean	Median	Std. Deviation	Variance	Skewness	Kurtosis
Asthma	126	117.738	95.0000	79.19426	6271.731	1.471	2.472
Wheezing	72	67.625	44.0000	69.18590	4786.688	2.630	6.658
Total	198	99.515	80.0000	79.30169	6288.759	1.633	2.674

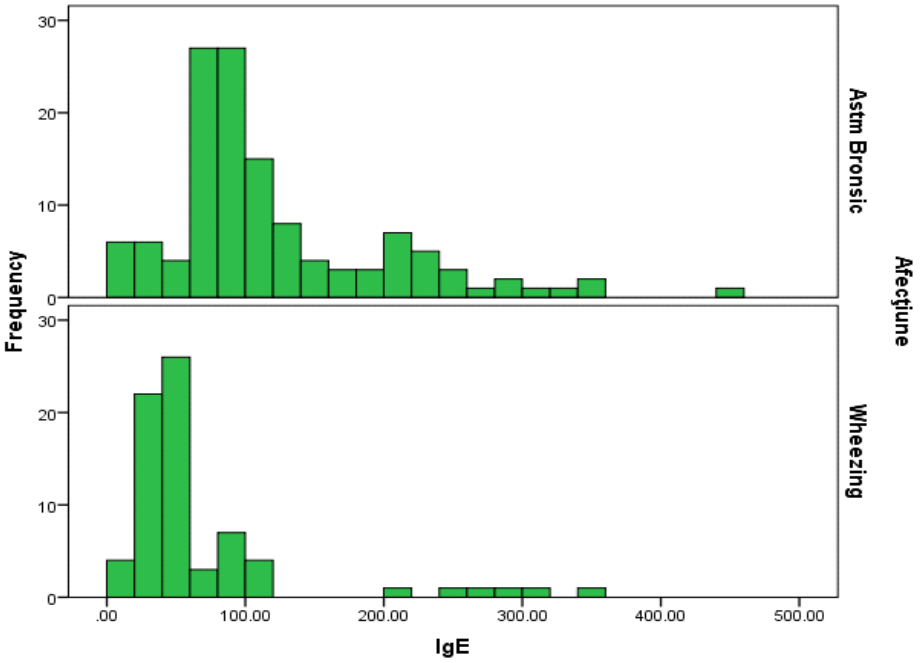

Figure 7 Distribution of IgE levels

In order to determine the existence of statistically significant associations between elevated IgE levels and asthma occurrence, the first step was data transformation into categorical data. Using the reference levels for the test and depending on the age of patients, it resulted that a total of 130 patients had levels above the normal range for this test.

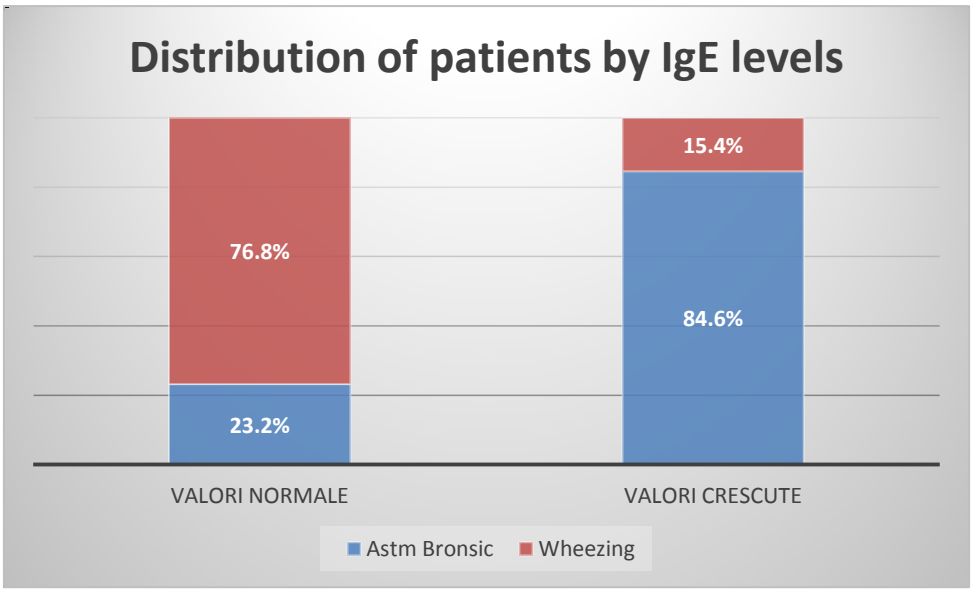

Figure 8 Distribution of patients by IgE levels

The figure nr.8 represents the distribution of patients by IgE levels for each condition. It can be observed an increased percentage of patients with normal levels in the condition called wheezing.

Chi-square test indicates a statistically significant association between the IgE levels and the occurrence of asthma at the patients followed in this study. ($p = 0.001$)

As follows, from 126 children diagnosed with asthma after the age of 5 by using the method of spirometry, 110 had high levels of IgE and 16 had normal levels. From a group of 73 children with wheezing, 53 had high levels of IgE and 20 normal ones. Specialty studies support the findings of my study. As follows, in 2012 [24] Keleş conducted a study on a group of 108 patients divided in 2 groups: patients with transient wheezing and persistent wheezing. It indicated that the average level of lgE in serum was 97.1% in the 1st group and 16.9% in the 2nd group with $p > 0.01$. These records indicate the necessity to dose the lgE in children with clinical occurrences of asthma, in order to determine the predictability of the evolution of these episodes.

IV.7 Classification of patients by eosinophils levels

The average percentage for the group of patients who developed asthma is 3.55% (standard deviation 3.52), while for the group of patients who have not been diagnosed with asthma, the average percentage of eosinophils was 5.67% (standard deviation 5.37).

By analyzing the distribution of levels (Figure nr.9), it can be noticed that the eosinophils levelsshow similar distributions, most cases showing levels below 5%.

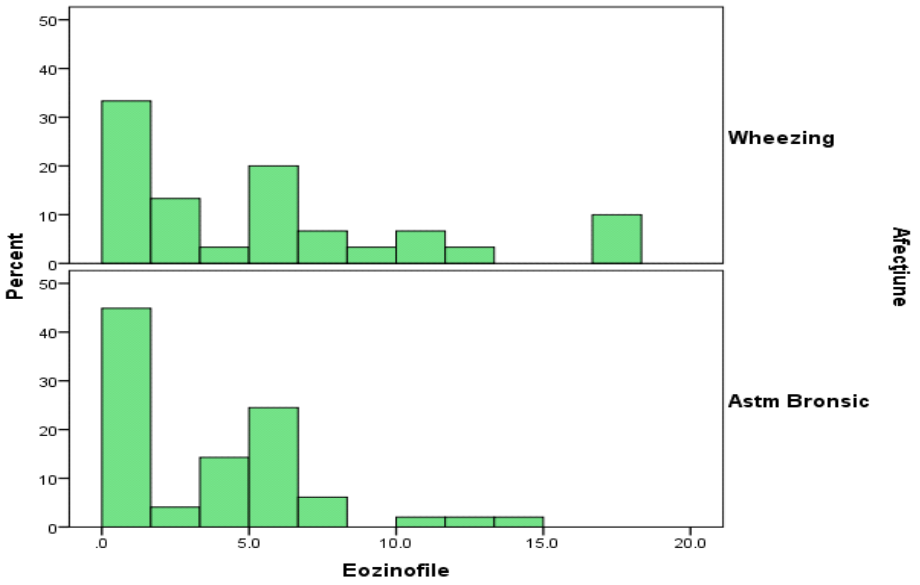

Figure 9 Classification of eosinophils levels

The two conditions indicate that the classification of the eosinophils levels differ significantly from a normal classification, which was demonstrated using the Shapiro-Wilk statistical test. (Table VII)

Table VII Testing the normality of classification of eosinophils levels

Teste de normalitate							
	Condition	Kolmogorov-Smirnova			Shapiro-Wilk		
		Statistic	df	p	Statistic	df	p
Eozinophils	Asthma	.192	49	.000	.860	49	.000
	Wheezing	.161	30	.046	.856	30	.001

Since this is a varied pathology which evolves with eosinophilia, its dosing in patients included in my study revealed no statistically significant importance in the evolution of children with wheezing towards asthma.

IV.8 Classification of patients by Phadiatop Infant test results

Phadiatop Infant test identified 67.2 % respectively 70.25% of patients who did not develop asthma and had a positive result in this test. (Table VIII)

Table VIII Classification of patients by Phadiatop Infant test results

Phadiatop Infant * Afecțiune Crosstabulation					
			Afecțiune		Total
			Asthma	Wheezing	
Phadiatop Infant	Negative	Count	36	21	57
		% within Lot	29.8%	32.8%	30.8%
	Pozitive	Count	85	43	128
		% within Lot	70.2%	67.2%	69.2%
Total		Count	121	64	185
		% within Lot	100.0%	100.0%	100.0%

Chi-square test has been used in order to determine if there is a statistically significant association between the Phadiatop Infant test result and the evolution of patients (to asthma or not). By analyzing the obtained results ($p = 0.668$), it cannot be confirmed with a sufficient degree of confidence that there is an association between the result of this test and the evolution of patients with wheezing towards asthma.

The Infant Phadiatop test may determine the atopic status in most children with asthma. Two Cochrane summaries related to how to avoid allergens revealed no benefit; they targeted domestic mites (49 trials with 2733 patients) and feline antigens (two trials with 57 patients). [25]

Many experts recommended the avoidance of allergens. It is also recommended by the guide issued by the British Thoracic Society / Scottish Intercollegiate Guidelines Network. If the family agrees, it can be resorted to the use of silicon linen or the removal of pets. In my study, the Infant Phadiatop test was positive at 70.25% of patients who developed asthma and at 67.2% of patients who did not develop asthma, demonstrating that the sensitivity to allergen can help to determine the atopic status of children in an early study.

IV.9. Classification of patients by C-Reactive Protein

We evaluated the CRP in the two groups of patients. The result indicates that the level of CRP is significantly higher in the patients who developed asthma in comparison to the ones who did not develop asthma ($p = 0.041$).

70% of patients who developed asthma had an elevated level of CRP. For the ones who did not develop asthma, the percentage was 72%. Therefore, there was no statistically significant difference between the two groups ($p = 0.80$). Therefore, it appears that level of CRP is increased to approximately 70% of patients in both groups, but for those who developed asthma, the level of CRP is significantly higher. (Figure 10)

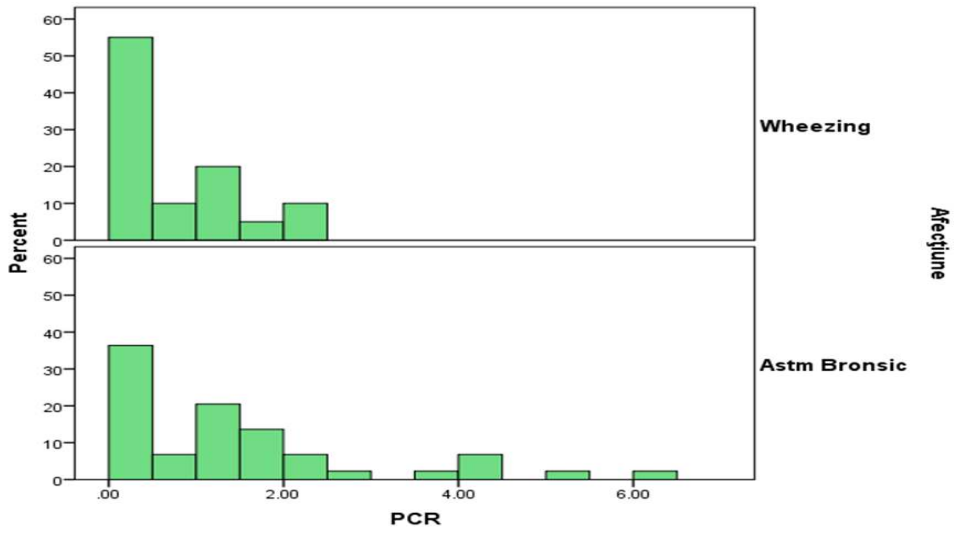

Figure 10 Classification of patients by C-Reactive Protein

VI.10 The distribution of patients according to the amount of fibrinogen

I analyzed the level of fibrinogen for the two groups. The result indicated that the patients who developed asthma had a level of fibrinogen significantly higher compared to the group of patients who did not develop asthma (p = 0.019).

85% of patients who developed asthma had an increased level of fibrinogen. For those who did not develop asthma, the percentage was 83%. Hence, there was no statistically significant difference between the two groups (p = 0.83).

Thusly, it was noticed that the level of fibrinogen was increased in approximately 80% of patients in both groups, but in those who developed asthma, this level was significantly higher. (Figure 11)

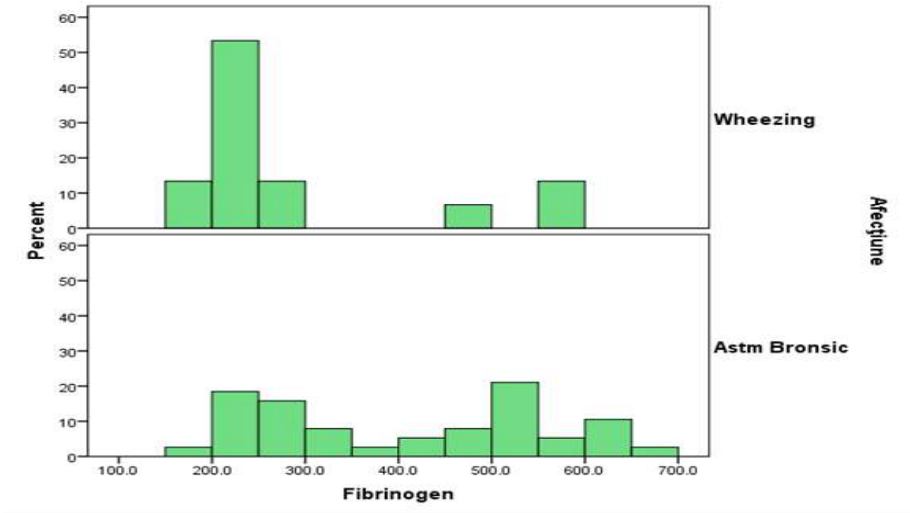

Figure 11 The distribution of patients according to the amount of fibrinogen

V. Possible screening tests

V.1 Eosinophilic cationic protein (ECP)

The ECP level in biological fluids represents an indication of the activation and the degranulation of eosinophils, currently used for clinical monitoring and the diagnosis of inflammatory diseases. Specialized studies indicated that the ECP determination in serum may be an objective indicator for clinical signs of asthma and it may indicate a potential pathophysiological axis based on the altering of the resistance of airways caused by eosinophils and the activity of the markers of eosinophil. The medical research will improve when it is confirmed that this axis plays a significant role in the pathophysiology of asthma.

Table IX represents the classification of cases; the limit level considered being 11 ug / l.

		Group		
		Disease	Healthy	Total
Cationic Eosinophilic Protein Result	Pozitive	110	50	160
	Negative	16	23	39
Total		126	73	199

By analyzing the table above (Table IX), it has been determined the sensitivity and specificity. Furthermore, the positive predictive level and negative predictive level are good indicators that we can report at.

ROC curve

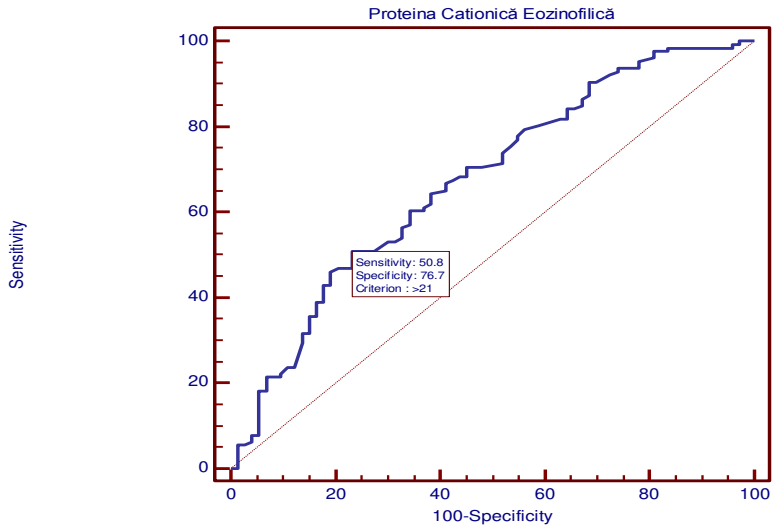

Figure 12 ROC Curve for ECP

V.2 Immunoglobulin E

The ROC figure (Receiver Operating Characteristic) displays the relationship between the real positive rate results(Sensitivity) by number of the false positives ones(100 - specificity), for different limit levels. Each point on the graph represents a pair of levels sensitivity / specificity specific for the limit levels.

A test is more accurate if both sensitivity and specificity are close to 100. Thus, an ideal test with sensitivity of 100 and specificity of 100 would pass through the upper left corner of the Figure.

The following Figure represents the ROC curve for IgE test conducted in patients from the group studied.

It is noted that optimum test results are obtained when the limit level of the test is 60 IU / mL. For this level, the calculated sensitivity is 83.7% and specificity 73.6%.

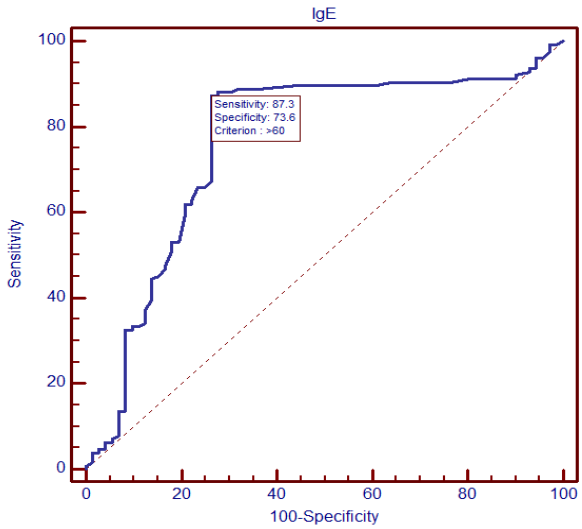

Figure 13 ROC Curve for IgE

The area underneath the ROC curve has a level of 0.76, which was statistically significantly different from the reference level (p <0.00001). This level indicates that the test has an average level in the screening of asthma in children with increased bronchial asthma reactivity, the result being highly statistically significant.

V.3 Eosinophilia

It is noted that optimum test results are obtained when in the determination of the eosinophils, the threshold level considered is <= 0.3. Patients with levels above 0.3 are at increased risk of developing asthma.

For this threshold, the sensitivity is 32 and the specificity is 93.1.

The area under the ROC curve is a measure of test accuracy. The greater the area is, the more accurate the test is. For the test of eosinophils, the area underneath the ROC curve is 0.62, indicating poor accuracy, the result being statistical significant (0.0434).

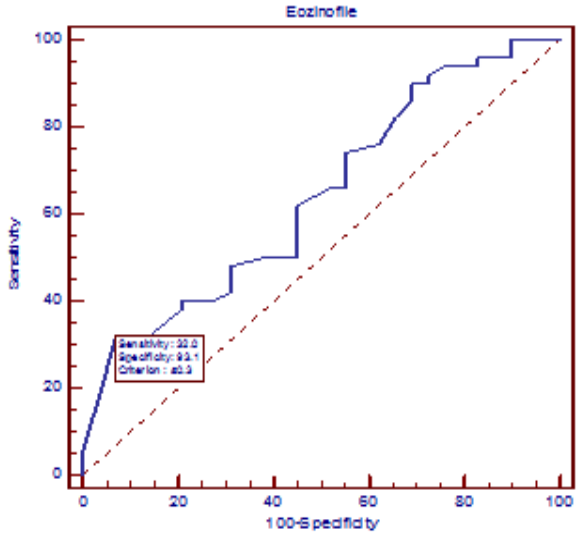

Figure 14 ROC Curve for Eosinophils

VI. CONCLUSIONS

1. Eosinophilic cationic protein level in serum may be a useful marker in the estimation of the eosinophilic inflammation in the airways in patients with bronchial hyperreactivity. My study suggests that eosinophilic inflammation is present from the first episodes of wheezing in children who will develop asthma at the age of 5.

By analyzing the data my study, the eosinophilic cationic protein dosage in serum has a sensitivity of 50.8 and a specificity of 76.7, for an eosinophilic cationic protein threshold level of 21µg / L.

Patients with higher eosinophilic cationic protein levels (21µg / L) had a significantly increased risk of being diagnosed with asthma after the age of 5.

2. The Immunoglobulin E level was high in most patients diagnosed with asthma using spirometry. The test has a specificity of 73.6 and a sensitivity of 83.7 for a threshold level of 60 IU / mL. The level of the area underneath the ROC curve indicates that the test has an average level in the screening of asthma in children with increased bronchial reactivity, the result being significantly high.

3. When the eosinophils level is below 0.3, the possibility that a patient is healthy is 93.1%.

4. During studying my group of patients in order to predict their evolution to asthma, I noticed that smoking is the main element that contributes to the respiratory symptoms. 54% of parents of children who developed asthma smoke and smoking during pregnancy was encountered in 50% of patients who developed asthma and 41.4% of patients who did not develop asthma.

5. The Phadiatop Infant test performed was positive in 70.25% of patients who developed asthma and in 67.2% of patients who did not develop asthma, demonstrating that allergen sensitization is another factor that contributes to outbreak of the respiratory pathologies, wheezing being the most common one.

6. The statistical analysis of the number of episodes of bronchial obstruction and asthma demonstrated that there is an association between the number of more than 3 episodes of bronchial obstruction in the past and subsequent diagnosis of asthma ($p < 0.001$).

7.Family atopia, the main criteria of asthma diagnosis, was present in over half of patients who had wheezing prior to developing asthma.

8. Patients who developed asthma were breastfed for a short time, for 2 months, while the group of patients who were hospitalized for wheezing but who did not develop asthma, they were breastfed for 3 months. This concludes to the belief that breastfeeding for a short period of time is a risk factor in the development of bronchial hyperreactivity.

9. In my study, the most frequently associated pathology is the allergic rhinitis (41% of children who developed asthma), followed by atopic dermatitis (19.7% of children).

10. Patients come with age specific risk factors for asthma, factors that if noticed and avoided on time, it would help reduce the severity of asthma and the frequency of episodes of bronchial obstruction. These are a few precaution measures in the early development of allergic sensitization and asthma: breastfeeding (for a longer period of time), adoption of necessary measures to reduce their exposure to allergens, avoiding smoking during pregnancy and passive smoking.

11. In order to prove the existence of bronchial hyperreactivity I evaluated the inflammation markers, whose values are statistically significant much higher in patients with asthma (85% of the cases), comparated with non-asthma subjects.

Ph.D. DISSERTATION ORIGINAL ELEMENTS

1. The main novelty of this dissertation is the ECP analysis as an important marker of the predictability of asthma, method currently little used.

2. The early prediction of the evolution of wheezing, by using the ECP which leads to a much earlier diagnosis of asthma (under the age of 5). Therefore, this allows to determine a more precise treatment plan created by needs of the patients and the improvement of the quality of their lives.

PERSPECTIVES OPENED BY PERSONAL RESEARCH

- The need to perform an allergy test during pregnancy in order to reduce the development of the allergic disease in children genetically predisposed;

- Special enrolment of the child with atopic risk in a primary prevention program at the family doctor's office, in collaboration with the pediatrician, allergist and parents in order to avoid the exposure to risk factors (passive smoking, excessive antibiotics, etc.);

- Further efforts should be taken to implement the monitoring of the inflammation of the airways by measuring the eosinophilic cationic protein ;

- The need to educate the family in order to prevent children's exposure to the risk factors of respiratory diseases.

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