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**VALIDATION OF ASTHMA SEVERITY AND ITS EVOLUTION
UNDER THE BACKGROUND THERAPY BY MEASURING
NITRIC OXIDE IN EXHALED AIR**

**PhD THESIS
(SUMMARY)**

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INTRODUCTION

The diagnosis of asthma and recurrent wheezing is often a challenge in pediatric practice.

Also, the need for a background, chronic, long-term therapy adjusted to individual particularities of the asthma child, properly monitored so as to reduce any adverse reactions, is a current goal.

Numerous studies in the literature have approached the use of some topical biomarkers that can more accurately and minimally invasively quantify the chronic inflammatory process at the level of the lower airways of children with asthma.

Based on these concerns in the pediatric respiratory medicine, this paper aims at demonstrating the utility of measuring the nitric oxide in the exhaled air of the asthma patient, in order to facilitate the diagnosis, monitoring and adjusting the background therapy according to certain peculiarities.

The thesis has 192 pages (of which, 121 pages represent personal research, 53 pages – the general part and 17 pages of general bibliography).

The paper is drawn up in a modern way (containing 111 figures and 70 tables in black and white and coloured) and is well documented (the bibliography includes 232 titles, over 30% in the last 5 years and 15% over the last 3 years).

The thesis is divided into 3 main parts: the theoretical (literature review), personal research and the bibliography.

PART I – LITERATURE REVIEW

The first part of the thesis consists of 8 chapters that deal with theoretical concepts related to asthma in children.

In Chapter I, entitled “**Asthma - etiology and immunological mechanisms**”, there are presented some fundamental concepts and modern theories of asthma genetics. A special attention was paid to genetic mutation in the HLA complex, those coding the synthesis of the receptor of high affinity for IgE (FcεR1), and respectively of the α/δ chains of the Th2 cells (TCR) receptor, as well as to the mutation of the cytokine gene group.

The same chapter refers to the immunological mechanisms of asthma, emphasizing the role of innate and acquired immunity and their components (respiratory epithelium, dendritic cells, macrophages, pulmonary surfactant).

Chapter II, entitled “**The inflammatory reaction in allergic asthma**” refers to some biomarkers specific of asthma. In addition to the role of inflammatory cells (Ly Th1 Ly Th2, Ly B, NK cells, macrophages, mast cells, eosinophils) and of cytokines secreted by them, mention is particularly made to the role of nitric oxide as a signal molecule, and the relationship between the increase of its concentration in the exhaled air and the eosinophilic inflammation at the level of lower airways in asthma patients.

Chapter III, called “**Asthma - classification and differential diagnosis**”, gives a brief overview of the classification of asthma and recurrent wheezing in children and some features of the clinical picture facilitating the disease diagnosis.

Chapter IV, entitled “**Asthma - clinical course and degrees of severity**” refers to the three types of clinical manifestations found in asthma (asthma attack, status asthmaticus and intercritical state), also presenting briefly the degrees of severity of asthma according to some international guidelines.

Chapter V, entitled “**Asthma - functional exploration**”, describes the main methods of diagnosis and monitoring of lung function (peakflowmetry, spirometry, total body plethysmography, challenge tests, tests with bronchodilators, diffusion capacity/transfer factor for CO) performance techniques and interpretation of results.

Chapter VI, entitled “**Bronchial asthma - airway inflammation exploration**”, refers to the main methods that highlight the chronic inflammation at the level of lower airways of the asthma patients: measuring the nitric oxide in the exhaled air, bronchoalveolar lavage and respiratory condensate. Within this chapter, special importance is given to the measuring

techniques, interpretation of results and the main advantages/disadvantages of measuring the nitric oxide in the exhaled air.

In **Chapter VII**, entitled “**Asthma - laboratory diagnosis and allergy exploration**”, there are presented the main clinical and laboratory methods meant to emphasize the presence of an allergic background in asthma children. There are mentioned and discussed issues related to sputum, nasal secretions and blood eosinophilia testing, allergy skin tests, dosing total and specific IgE and eosinophilic cationic protein.

Chapter VIII, entitled “**Defining controlled asthma and intercritical anti-inflammatory background therapy of asthma**” mentions the control criteria of asthma and treatment protocol, emphasizing the main classes of drugs used as anti-inflammatory background therapy: inhaled corticosteroids and inhibitors of leukotrienes.

PART II – PERSONAL RESEARCH

The premises of the research are based on the concerns in the field of pediatric respiratory medicine regarding the diagnosis and monitoring of inflammatory process of asthma in children by a simple, non-invasive, reproducible method, respectively by measuring the nitric oxide in the exhaled air.

Also, individualizing the background therapy according to certain demographic, clinical, spirometric parameters and, especially, according to the values of the nitric oxide in the exhaled air, represented one of the targets of this research.

The increased prevalence of asthma and recurrent wheezing in pediatric age population in Romania is a worrying factor. Asthma is considered the most common chronic disease in children, particularly in school-aged children.

The scale of this phenomenon is best reflected in the expenditure allocated for hospitalization and treatment of these patients, but also in the indirect costs arising from truancy.

The marked increase in the variety and severity of allergic diseases will likely lead in the not too distant future, to a growing number of patients diagnosed with atopic asthma.

That is why it is necessary to develop modern methods to better quantify the eosinophilic inflammation of the respiratory tract in asthma children, this necessity representing one of the motivations of the current study.

Important **objectives** were formulated in this research aiming at establishing a correlation between the amount of nitric oxide in exhaled air and various parameters (demographic, clinical, spirometric) in children who had as background therapy an anti-inflammatory drug (inhaled corticosteroid) or a combination of two anti-inflammatory drugs (inhaled corticosteroid associated with leukotriene inhibitor).

In terms of the **methodology of the study**, the study included 90 children with asthma or recurrent wheezing, in the evidence of the “Center for Laboratory and Clinical Research in the Field of Pediatric Respiratory Medicine” (Pediatric Clinic of Sibiu) between 2013 and 2014.

It was a longitudinal, prospective, analytical, comparative, “exposed/unexposed” observational, randomized study.

The 90 patients were divided into two equal groups: a group under anti-inflammatory background therapy with inhaled corticosteroids, the other group under background therapy with inhaled corticosteroids associated with leukotriene inhibitors.

Patients were enrolled in the study according to specific inclusion and exclusion criteria.

The study included male and female children, aged 6-18 years old, who came from rural or urban environment.

The study also took into account the pathological personal and family history of atopy, the values of total IgE and blood eosinophilia, the data being taken from the patient record files.

The children were classified in certain stages of severity of asthma/recurrent wheezing, for which they had been receiving “controller” treatment for about 3 months.

A first assessment took place after 3 months of background therapy, and the second, 3 months later when certain parameters were analysed, both within the two subgroups, and within the total group.

Clinically, cough, dyspnea and wheezing have been analysed, which occurred in different circumstances (diurnal, nocturnal and on exertion).

In terms of lung function, FEV₁ was measured by spirometry and FeNO in the exhaled air.

Spirometry was performed with the spirometer belonging to the Pediatric Clinic of Sibiu and FeNO measurement in the exhaled air was performed with NObreath® portable device, which belonged to the “Center for Laboratory and Clinical Research in the Field of Pediatric Respiratory Medicine” in Sibiu.

The concentration of nitric oxide in the exhaled air is expressed in ppb (parts per billion), and interpretation of results obtained after measurement have met the criteria formulated by the American Thoracic Society:

- FeNO > 35 ppb = eosinophilic inflammation and responsiveness to inhaled corticosteroids;
- FeNO between 20-35 ppb - interpreted according to the clinical context;
- FeNO < 20 ppb = normal values = eosinophilic inflammation and responsiveness to inhaled corticosteroids less probable;
- Increase of FeNO by 20% prior to the previous measurement in patients with initial FeNO > 35 ppb = weak response under the background therapy;

Statistical processing was performed using SPSS v. 16.

For characterization of group homogeneity, I analysed the distribution by gender and area of origin, age, pathological personal or family history of atopy.

In the chapter “**Results and Discussions**”, in order to demonstrate the benefits of measuring the nitric oxide in the exhaled air of children with asthma or recurrent wheezing, as part of monitoring the development and modulation of background therapy, several parameters (demographic, clinical, spirometric) were aimed at in dynamics (at 3 months and 6 months after onset of background treatment) in the two groups (the group with ILT and the group without ILT), which correlated with FeNO value. The results obtained were compared with the results of other studies on the same topic found in the literature.

Thus, there have been made correlations between FeNO values and gender, age group, area of origin, pathological personal or family history of atopy and the values of serum total IgE and blood eosinophilia.

In the studied groups, there were also analysed, FeNO fluctuations according to the anti-inflammatory treatment, correlations between FeNO and FEV₁ at the first measurement, respectively FeNO and FEV₁ at the second measurement, evolution in dynamics of FeNO values compared with the evolution in dynamics of FEV₁ values.

At 3 and 6 months after treatment, in the studied groups, the impact of background inflammatory therapy on FeNO has been analysed, but also on specific symptoms of asthma (shortness of breath, coughing, wheezing) and the circumstances surrounding the appearance thereof (diurnal, night, on exertion). There were also analysed the progression of symptomatology under the background therapy.

The research also focused on other important aspects of asthma/recurrent wheezing in the pediatric patient, namely the distribution of cases by type of asthma (intermittent, mild persistent, moderate persistent) and gender, age, area of origin, personal or family history of atopy.

There have been also examined the distribution of pulmonary function laboratory investigations, depending on the type of asthma and treatment.

The findings were presented in figures and tables, and the following is a summary of them:

- The studied groups were homogeneous in terms of correlations between gender and area of origin, age, family history association of atopy, positive personal history for allergies.
- In the studied groups, it is shown that males are more often associated with elevated FeNO, while most girls show normal levels of FeNO.
- The introduction of ILT in the background therapy in the male asthma patients may lead to a fall in FeNO values.
- Analysing the distribution of patients by age groups and FeNO reveals that most subjects with increased FeNO are over 10 years old.
- In the group with ILT, after 3 months of treatment, there was an association between the age group > 10 years and elevated FeNO values and between the age group ≤ 10 years and FeNO normal values, the statistical difference was significant.
- In patients with ILT, there is a beneficial effect of this anti-inflammatory drug especially in the age group > 14 years, the percentage of subjects aged > 14 years with high FeNO in the group with ILT dropping after 6 months of treatment compared to 3 months, more than in the other age groups.
- In the studied groups, there could not have been revealed any statistically significant association between FeNO values and area of origin after 3 months, respectively after 6 months of treatment.
- In the studied groups, the number of patients coming from rural areas was lower than those in urban areas.
- It may be noted, however, that the percentage of patients with increased FeNO is higher in patients from urban areas.
- ILT introduction in the background therapy has led to a fall in FeNO values after 6 months of treatment especially in subjects from urban areas, but this decrease was insignificant.
- In the studied groups, there could not have been revealed any associations between FeNO values and family history of atopy.
- ILT association with background therapy in subjects with a family history of atopy seems to result in a decrease of FeNO values after 6 months as compared to the measurement made after 3 months of treatment.

- ILT association with background therapy in subjects with personal pathological history of atopy, elevated total IgE and high values of eosinophils does not significantly influence FeNO values.
- The percentage of patients with increased FeNO associating high levels of total IgE has increased both in the group with ILT and in the group without ILT after 6 months of treatment compared with the percentage registered after 3 months of treatment.
- The percentage of patients with increased FeNO associating high levels of blood eosinophilia has increased both, in the group with ILT and in the group without ILT after 6 months of treatment compared to the percentage after 3 months of treatment.
- Analysis of the groups under study demonstrated a statistically insignificant difference regarding FEV₁ fluctuations correlated with FeNO.
- In the subgroup with ILT, there was noticed a slight improvement in lung function at 6 months compared to 3 months in terms of FEV₁, but it was not correlated with a decrease in subjects with increased FeNO.
- There is a statistically significant difference between the two groups (with and without ILT), founding an association of cough with FeNO values only at 6 months and only in the group without ILT.
- Regarding dyspnea or wheezing and FeNO values, there is no statistically significant difference between the two groups (with and without ILT).
- ILT association with the background treatment of asthma/recurrent wheezing is accompanied by an improvement in symptoms on exertion, but it was not statistically significant.
- At the dynamic analysis (after 3 and then after 6 months of treatment), the introduction of ILT in the background therapy seems not to have had a significant impact on symptoms (cough, dyspnea, wheezing), occurred in different circumstances (diurnal, nocturnal, on exertion), in other words, one can say that there is a significant association between FeNO and asthma symptoms.
- One cannot say that there is an association between the type of asthma and gender, area of origin, personal or family history of atopy.
- There is a slight predominance of recurrent wheezing in boys and a slight predominance of asthma in girls.
- Both in rural and in urban areas, there is a slight predominance of recurrent wheezing.

- At the level of the total group, one can say that there is a statistically significant difference between the type of asthma and FEV₁, namely in moderate asthma, FEV₁ average is less compared to that of recurrent wheezing.
- It can be said that there is a statistically significant difference in the total group, between the type of asthma and FeNO, namely in moderate asthma, FeNO average is higher than that in mild asthma.
- In patients with normal FeNO, regardless of background therapy, it can be said that there is a statistically significant difference between the type of asthma and FEV₁, namely in moderate asthma FEV₁ average is less compared to that in recurrent wheezing.
- In patients with normal FeNO, regardless of background therapy, it can be said that there is a statistically significant difference between the type of asthma and total IgE, namely in moderate asthma, the average is higher compared to that in wheezing recurrent.
- In the subgroup without ILT, one can say that there is a statistically significant difference between the type of asthma and FeNO, so in moderate asthma, FeNO average is higher compared to that in recurrent wheezing.
- In the subgroup with ILT and normal FENO, it can be said that there is a statistically significant difference between total IgE and type of asthma, namely that in moderate asthma, total IgE average is much higher compared to that in recurrent wheezing.

Interpretation of the results of research by correlating the data obtained in the literature, in studies dealing with the measurement of nitric oxide in the exhaled air of asthma children and its role in monitoring the progress and in modulating the background therapy has led to some important **conclusions**:

- Male gender is more frequently associated with elevated FeNO than female gender.
- Introduction of ILT in the background therapy in asthma male subjects may lead to a fall in FeNO values.
- There is an association between the age group > 10 years old and elevated FeNO values and between the age group ≤ 10 years old and normal FeNO values.
- Introduction of ILT in the background therapy resulted in an insignificant decrease in the values of FeNO, and especially in the age group > 14 years old.
- ILT association with the background therapy in the subjects in urban areas and in those with a family history of atopy has led to a decrease in FeNO, which was insignificant.

- ILT association with the background therapy in subjects with personal history of atopy, elevated total IgE and high eosinophils does not significantly influence FeNO values.
- There is no significant correlation between FeNO values and FEV₁.
- There is a statistically significant difference between the two groups (with and without ILT) regarding the association between the type of cough and FeNO values only at 6 months and only in the group without ILT.
- There is no statistically significant difference between the two groups (with and without ILT) regarding dyspnea or wheezing and FeNO values.
- ILT association with CSI is accompanied by an insignificant improvement of symptoms on physical exercises.

As a **personal contribution**, through this study, I tried to emphasize the importance of completing the protocol for diagnosis and monitoring of asthma/ recurrent wheezing through the determination of nitric oxide (FeNO) in the exhaled air, and also, it is the first work published in our geographical area, which aims at demonstrating that modulating the inflammatory background therapy of asthma/recurrent wheezing can be achieved by measuring the nitric oxide in the exhaled air.

The originality of the study lies in the complexity of the correlations performed in dynamics between FeNO and symptoms occurred in different circumstances or between FeNO and FEV₁, but also in considering the weight of other risk factors in asthma or recurrent wheezing (gender, age group, origin environment, association of personal and family history of allergy, the values of total IgE and blood eosinophilia).

In terms of **proposals** emerging from this study, I mention the following:

- taking into account the measurement of FeNO in the exhaled air in the diagnostic protocol, in monitoring the progress and in modulating the background therapy in asthma and recurrent wheezing.
- considering the decision to introduce ILT in the background treatment of asthma and recurrent wheezing, especially in male subjects, in patients from urban areas, in older age groups, in the children with a personal history of atopy and in those in whom the symptoms during exercise represent a dominant component of the clinical picture.