″Lucian Blaga″ University of Sibiu

Doctoral Thesis

The role of ultrasensitive „C" reactive protein in assessing childhood asthma

ABSTRACT

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Introduction

Medicine is one of the most changeable scientific areas, continuously updating and tinting. This is due primarily to the fact that medicine is centered on man which represents a whole universe of unknowns. When referring to the pediatric patient, valences are enriched even further owing to the child transformations before our eyes.

Worldwide over 334 million people suffer from asthma, being 14th place in importance when taking into account it′s expansion and duration. Pediatric asthma prevalence is increasing, 14% of children presenting asthma symptoms, the most affected group being formed of children between 10 and 14 years of age.

Asthma is a heterogeneous entity both in terms of clinical, pathophysiology and inflamometry, the list of trigger factors continues to expand in the context of urbanization and industrialization. The most important and up-to-date challenge is to classify recurrent wheezing and asthma. Older classifications capture only limited aspects of the disease that are not able to provide the actual size of asthma in terms of pathophysiology, variants of inflammation biomarkers, evolutivity, response to therapy and prognosis. Therefore, asthma should be regarded mostly as a syndrome rather than a disease.

Assessment of the asthmatic inflammation, localized or systemic, is an important goal. Some markers have proven valuable to this purpose. For this we benefit of the progress made in cellular and molecular medicine, genetics, histopathology and ,,omics" sciences. The exact way in which biomarkers will find their place in practice remains to be held. Using biomarkers as inflamometric tools certain models of inflammation can be identified which can serve later in monitoring and therapy.

Identifying the aspects that can influence the disease make pediatric asthma even more interesting. However, the correct approach to pediatric asthma may confer advantages even to the adult asthma.

In the **GENERAL PART**, over two chapters the updated definitions, epidemiological data, classifications related to asthma and recurrent wheezing are presented along with the factors involved in the pathogenesis of asthma. New and useful concepts are introduced such as: asthma phenotype, asthmatic endotype, hierarchical cluster creating a shortsightedness overview of asthma. Recent classifications of recurrent wheezing and asthma based on various criteria such as: triggering factor, natural history, asthmatic phenotype, asthmatic endotype, disease severity, asthma control and therapy response are detailed. The second chapter focuses on the factors involved in the pathogenesis of asthma, starting with genetic and continuing with environmental factors that determine altered immune responses and consequent inflammation.

Asthmatic inflammation involve all the components of immunity in different degrees. All the components of innate immunity participate in the pathogenesis of asthma such as epithelial cells, lung surfactant, immunocompetent cells - dendritic cells, alveolar macrophages, natural killer cells, mast cells and neutrophils, as well as their mediators.

The two distinct facets of acquired immunity, humoral immunity performed by B lymphocytes and immunglobulins and cellular immunity involving T lymphocytes, with their different subtypes, are an important part in the pathogenesis of asthma. The central element is the imbalance Ly Th1 and Th2 in favor of LyTh2, but other subtypes are also involved, such as lymphocytes Th17 and Th9. Cytokine environment directs polarization to different subsets of T lymphocytes responsible for different aspects of the immune response.

SPECIAL SECTION spans six chapters, the first being reserved to inflammatory reaction and then are presented in order, relevant issues related to personal research: motivation, goals and research methodology, results, discussion and conclusions highlighted in the study, and recommendations that can be drawn. The most important elements related to inflammation constitute the premises for research. Aspects such as: types of inflammation, mechanisms involved in inflammation, general changes in the inflammation - classification and dynamic of acute phase reactants, characteristic features of inflammation are approached. Particularities of inflammatory reaction in asthma are detailed in a separate section. The last section is reserved for chronic low grade systemic inflammation associated with various entities (age, type I diabetes, obesity, irritable bowel syndrome, psychological stress) and associated to asthma. Identification of chronic low grade systemic inflammation is possible using biomarkers such as ultrasensitive

C-reactive protein (hs-CRP). Recent studies (2005-2015) describe the defining features of asthma correlated with hs-CRP. Their conclusions were later systematized, focusing on controversial issues identified in the literature, such as the association between hs-CRP and bronchial hyperreactivity, lung function and total IgE.

Personal research

This research aims to contribute to the evaluation of chronic inflammatory status in children with asthma and recurrent wheezing, by determining hs-CRP, with the declared purpose of identifying pediatric particularities and even hightlight elements of diagnosis, therapy and ways of progression.

Data from international studies on low-grade inflammation in children are limited to a few issues such as overweight and obesity, metabolic syndrome, association with asthma, while in Romania no major study approached this subject.

The main objective of the present research was to evaluate the ultrasensitive "C" reactive protein (hs-CRP) as a marker of low – grade systemic chronic inflammation in children with recurrent wheezing and asthma admitted in the study group vs. children in the control group without acute or chronic inflammatory pathology, asthma or recurrent wheezing.

As secondary objectives we pursued the assessment of other aspects that might be relevant in association with ultrasensitive CRP, such as: individual factors (age, gender, origin, vaccination, prematurity, passive smoking), particularities (atopy in family and personal history, presence of rickets, IgE values), therapy administered prior to the testing and associated factors (anemia and changes in nutritional status).

Methodology

The study underpinning the research was a prospective study, conducted at the Clinical Hospital of Pediatrics of Sibiu, for 17 months, during October 2013 - February 2015 and is based on two groups, a study group consisting of 92 children diagnosed with recurrent wheezing and asthma and a control group consisting of 84 healthy children. The patients included in the study were aged between six months and 18 years.

The inclusion criteria for patients in the study group were represented by: diagnosis of recurrent wheezing or asthma, "C" reactive protein in normal ranges, absence of infectious

diseases in recent history, absence of chronic diseases in personal history (diabetes mellitus, inflammatory bowel disease, immune deficiencies). The control group included healthy children without chronic or acute pathology in recent history and with normal levels of "C" reactive protein. For both groups we conducted anamnestic, clinical and biological assesssment.

Anamnestic assessment targeted data collection related to family history of atopy, physiological personal history, personal history of atopy, history of the chronic respiratory symptoms, data of chronic therapy (subjects without previous therapy, subjects treated with inhaled corticosteroids (CSI), subjects treated with montelukast and one subject treated with the combination of inhaled corticosteroids and montelukast).

Clinical evaluation permited considerations on nutritional status, presence and severity of respiratory symptoms, and biological evaluation with the assessment of "C" reactive protein (CRP), ultrasenzitive "C" reactive protein (hs-CRP), blood eosinophil percentage, hemoglobin, total E immunglobulins (IgE total).

For both C" reactive protein and ultrasenzitive "C" reactive protein we used an immunoturbidimetric method using KonelabT kit series.

Expected values of hs-CRP by immunoturbidimetric method are below 3 m̃gl. According to the Center for Disease Control and Prevention the reference range for hs-CRP in healthy individuals varies between 0.08 to 0.5 mg / l.

The titer of IgE was determined by using an enzyme immunoassay.

Data was collected in Excel format and then statistically analyzed using SPSS, version 20. Statistical tests were used taking into account the level of significance / error probability of $p < 0.05$. Multiple elements were analyzed by descriptive statistics, the quantitative data were subjected to Kolmogorov-Smirnov test, parametric tests were used further such as: Student t-test, Mann-Whitney test, ANOVA test, Kruskal-Wallis test. In order to determine the correlation and coefficient of determination, was calculated Pearson correlation coefficient (r). For qualitative data were used contingency tables.

Graphical representation of analyzed data was made using different types of charts.

Results and discussions

Comparative results between groups depending on the unquantifiable characteristics

This study aimed to identify those conditions or parameters specific to pediatric patient in order to use the ultrasenzitive "C" reactive protein, so studied in adult pathology, as a marker for assessing and monitoring pediatric asthma and recurrent wheezing. Some of the variables that we studied have been studied in adults and were described in this study hightlighting the particularities associated to pediatric age. This research was a good opportunity to reiterate the idea that the assessment of specified issues (proper classification, individualized therapy) can have positive consequences including on adult asthma.

From the demographic data we can conclude that male subjects had higher representation, with more than half of all enrolled subjects. Two thirds of asthmatic patients were 0-6 years of age, more than half of them belonging to the male sex.

The conclusions are consistent with other studies confirming that the prevalence and incidence of asthma patients are sex – dependent. Higher values of these parameters are found for males before puberty - teens, and reverses in favor of females afterwards. The explanations are related to anatomical, immunological and hormonal features.

Family allergies were absent in most subjects enrolled in the study, only one-tenth of asthmatics enrolled recognizing positive history.

Bronchial asthma was present in all patients in the study group who had a positive family history for allergy. The conclusions are similar to those reported in different studies that associate parental allergies with an increased risk of allergy in children. However, the literature shows that certain types of allergy have a stronger hereditary component than others, some scientists even suggesting sex dependence of the allergy and asthma offspring.

There is no statistically significant difference between the two groups studied if we refer to all types of allergies present in personal history.

One third of asthmatic patients with personal history of allergy presents a positive family history. Asthmatic patients with a history of positive allergy have higher values of hs-CRP compared to asthmatics who have a history of allergy but there was no significant statistical difference. The studies linking hs-CRP with personal allergies are inconclusive.

There was observed a statistically significant difference between the groups according to the state of nutrition, a tenth of the included subjects was beyond the reference range for weight. The mean value of hs-CRP in subjects with asthma was higher compared to the control group, with no correlation with weight of the subjects.

Regarding the vaccination status of patients we found is a statistically significant difference between the two groups. We did not make any considerations concerning the relationship between vaccination status and hs-CRP, this fact was not a subject for any study.

Most of the subjects declare that they have good living conditions with significant difference between the two groups. Most patients in the study group come from rural areas, in contrast with data reported in the literature that suggests the protective effect exerted by rural environment regarding asthma. Comparing data of maternal smoking during pregnancy between the two groups does not reveal statistically significant difference between them. In both study group and control group considered together there was a statistically significant difference between smoking and nonsmoking mothers. Mean value of hs-CRP for the children of the smoking mothers was higher than for the children of nonsmoking mothers.

Literature data are consistent on maternal smoking during pregnancy, especially during the first trimester. Smoking during pregnancy is considered a predisposing factor for early onset of asthma, with ongoing effects until adolescence.

Comparative results on groups and correlation with ultrasensitive "C" reactive protein

• Mean value for "C" reactive protein was higher in the study group compared to the control group, with no statistically significant difference between the two groups.

• Mean value for ultrasensitive "C" reactive protein was higher for the study group compared to the control group, with statistically significant difference between the two groups.

• Age does not influence hs-CRP values and there were no statistically significant differences between groups.

• The study highlights the significant difference between the two groups in terms of the percentage of eosinophils, eosinophil levels in the study group were significantly lower than in control group.

• IgE values were significantly higher in the study group than in the control group.

• Therapy administered prior to the assessment affects hs-CRP values. Mean value of hs-CRP in patients treated with inhaled corticosteroids average was significantly lower than for the subjects that did not receive prior therapy, consistent with the data in the literature.

• The mean value of hs-CRP were significantly lower in children treated with montelukast compared to untreated. Data associating hs-CRP with leukotriene inhibitors are sparse and inconclusive.

• Hs-CRP values were lower for those patients who had respiratory failure at the time of admission and for the patients with stable asthma, without significant differences between subgroups of patients. The results are consistent with those reported in the literature stating that hs-CRP levels are higher during asthma exacerbations compared with those with stable asthma

• The mean value of hs-CRP in children exposed to passive smoking was higher than the values identified in subjects with no passive exposure to cigarette smoke, without statistical significance. The literature correlates passive smoking with worsening symptoms, increasing the number of asthma exacerbations and poor control of asthma.

• The study concludes that a fifth of asthmatic patients included in the study and ten times fewer subjects in the control group were born prematurely, identifying statistically significant difference between groups. Within the study group, hs-CRP mean values were higher in patients born prematurely, without showing statistically significant difference. The results are consistent with the literature, prematurity being considered an independent risk factor for the development of recurrent wheezing and asthma related to the degree of prematurity.

• There was no significant statistically differences between the two groups concerning rickets prevention. Two-thirds of asthmatic subjects and more than half of the control group received proper prophylaxis of rickets. Mean value of hs-CRP in subjects with proper prophylaxis for rickets in the study and control group considered together was lower than in those with incomplete or absent prevention of rickets. Studies correlate low levels of vitamin D with asthma. Vitamin D deficiency in the mother causes structural alterations during lung development. In patients with asthma, vitamin D deficiency is associated with altered lung function, with increased airway responsiveness and reduced response to corticosteroids.

• One third of the patients in the study had anemia at the time of inclusion.

The literature shows that hemoglobin levels are associated with an increased incidence of the allergy and asthma and also that patients with allergies or asthma have a higher prevalence of anemia than healthy children.

Conclusion

1. In the structure of the studied population prevailed male subjects, patients under 6 years of age, and those from rural areas.

2. Considering all types of allergy, there is no statistically significant difference between the two groups. Family allergies were revealed at a tenth of asthmatic subjects studied and in all cases, asthma was part of family history, personal allergies are found in a quarter of the study subjects, the most frequent were drug allergies and a lower percentage of subjects presents both personal and family history of allergy.

3. The majority of patients evaluated in the study were fully and properly vaccinated, they declared good living conditions, with statistically significant difference compared to healthy children evaluated.

4. The study concludes that only a small percentage of mothers declared they have smoked during pregnancy, a quarter of asthmatics were exposed to passive smoking, without showing statistically significant difference between groups.

The conclusions highlighted by biological evaluation of patients with recurrent wheezing and asthma in the study can be summarized as follows:

5. Mean value of "C" reactive protein was higher in the study group, with no statistically significant difference compared to the control group, demonstrating that the "C" reactive protein is not useful in assessing asthma and low-grade inflammation.

6. This study confirms that serum levels of hs-CRP were significantly higher in children with asthma or recurrent wheezing compared to healthy children, mean value of ultrasensitive "C" reactive protein for study group is significantly higher than in the control group.

7. Three-quarters of hs-CRP values of asthmatic subjects is above the maximum reference range, demonstrating the presence of low-grade chronic inflammation in asthma.

8. Mean total IgE titer was four times higher for the study group compared with controls, with statistically significant difference between the two groups.

9. The percentage of eosinophils was lower in patients with recurrent wheezing and asthma compared to healthy subjects, the difference being statistically significant and in patients with no CSI treatment were found positive correlations between hs-CRP values and the percentage of eosinophils.

10. Asthmatic patients with personal history of allergies, those in proximity of a exacerbation and those exposed to passive smoking had higher value of hs-CRP compared to those with stable asthma and with no exposure to passive smoking.

11. Patients with asthma or recurrent wheezing, those with prenatal exposure to cigarette smoke had significantly higher hs-CRP value compared with healthy children and those that were not exposed to prenatal cigarette smoke.

12. Asthmatic patients with previous therapy (CSI or montelukast) had significantly lower hs-CRP value compared to asthmatic with no previous controller therapy.

13. Hs-CRP value was lower, but not statistically significan, in patients treated with inhaled corticosteroids compared with patients treated with montelukast.

14. Hs-CRP values are not influenced by the age and the sex of the patient did not seem to affect the relationship between various parameters: for asthmatic patients positive correlations are more common in women (PCR and hs-CRP values of hs-CRP and the titre of total IgE) and for healthy children positive correlations are more common in males (PCR hs-CRP and percentage of eosinophils and IgE titers total). Also, the deficiency of vitamin D 3 does not influence the value of hs-CRP.

15. At the time of enrollment one third of the patients experienced anemia, data correlated with the literature considering that anemia is a risk factor for asthma.

16. Ultrasensitive "C" reactive protein is a valuable biomarker for assessing childhood asthma, as demonstrated by other studies. This research proposes the identification of the relationship between hs-CRP and other parameters in order to reveal a new asthmatic endotype.

Global assessment of the study

This research is aligned to global concerns related to systemic inflammation in asthma. However, most of the studies were conducted in adult patients, only a small part of them aimed the children, so this study is intended as a contribution meant to enrich the information for the pediatric population.

In Romania is one of the few studies assessing asthma in terms of systemic inflammation, national studies evaluating asthma through the determination of hs-CRP are few and inconclusive. This research is the first national attempt to evaluate children with recurrent wheezing and asthma by hs-CRP.

The novelty and originality of the research are:

- assessing the asthmatic child using a biomarker mostly used in adult pathology, especially in relation to diseases involving the body as a whole;
- complex and novel correlations between hs-CRP and various parameters (personal and family history of atopy, living conditions, vaccination status, prematurity, association with anemia or rickets);
- identification of correlations between hs-CRP and serum eosinophilia;
- sumarizing data from recent literature correlating hs-CRP with different defining aspect of asthma.

All aspects analyzed in this research converge on the same conclusion that the use of hs-CRP as a biomarker in assessing asthmatic child is particularly useful. The information provided is precious, hs-CRP brings valuable information on the past and the present of asthmatic inflammation, but it is also a tool for forecasting future exacerbations.

Reserves related to the current determination in practice can be linked to financial costs that are still discouraging.

Recommendations

Some aspects may be practical applications for asthmatic child:

- hs-CRP determination is not limited by age or sex, can be used in most children without requiring adjustments;
- benefits outweigh the costs (cost dosage, costs for hospitalization, school absenteeism etc.);
- no other biomarker catches so intimate the local and systemic inflammatory process in asthma, so routine measurement of hs-CRP provides additional information and completes the information brought by other biomarkers in order to make a "model of inflammation" for each patient;
- regular testing could provide the data needed for individualized therapy;
- further studies dedicated to asthmatic children are needed to show how hs-CRP can contribute to asthma management.

Key words: asthma, inflammation, ultrasensitive „C" reactive protein

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