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# PhD THESIS

## PREDICTIVE ALLELES OF THE RISK OF EXACERBATION IN ALLERGIC BRONCHIAL ASTHMA – MBL2D AND MBL2H

## A B S T R A C T

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# ABSTRACT

Bronchial asthma is a subject of controversy in the specialized literature, being defined by the Global Initiative for Asthma (GINA) as "a heterogeneous disease, characterized by chronic inflammation of the airways", with excessive production of immunoglobulin E (IgE) and influx of leukocytes, especially eosinophils.

This topic is very important for our study, which has as its main theme the analysis of genetic changes found in allergic bronchial asthma in children and the examination of the role played by the mannose-binding lectin (MBL) gene and the role of the MBL2D and MBL2H alleles in the pathophysiology of acute attacks of the disease.

The subject of our research is of global interest, bronchial asthma being a disease specific to industrially developed countries (its prevalence being 7% in France, 8% in the United States and 15-18% in England). For this reason, current researchesare focused on the discovery of genetic tests for early diagnosis and more effective personalized treatment of bronchial asthma. The MBL gene and its alleles, MBL2D and MBL2H, are of global interest, and more and more studies are investigating their role as atrigger factor in asthma and their role in disease progression. It is known about the glycoprotein secreted by the MBL gene, called mannose-binding lectin, that it intervenes in the opsonization process and is the first component of complement on the lectin pathway, and MBL insufficiency causes increased susceptibility to infections and atopy. In Romania, there are no approved screening tests to help correctly assess the prevalence of asthma, the disease being underdiagnosed. It is estimated that in Romania there are over a million asthmatics, although we figure with a prevalence of 3-6 people per 100,000 inhabitants. Also, an increasingly high incidence of bronchial asthma is observed in children from urban areas and of problematic-severe asthma (3.7% versus 1.5%).

Our study aims to determine the risk of exacerbation in allergic asthma in children and analyse the clinical relevance of serum MBL level and the presence of MBL2D and MBL2H alleles in the pathophysiology of acute attacks of the disease. Determination of MBL gene expression and the presence of MBL2D and MBL2H alleles in patients under 18 years may be developed in the future as a diagnostic and

possibly prognostic test in asthma. Patients who had the MBL2D and MBL2H alleles in the genome had a higher risk for severe exacerbation episodes.

Current studies are analysing the genetic changes found in allergic asthma and examining the role played by the MBL gene and the MBL2D and MBL2H alleles in the pathophysiology of acute exacerbations of the disease.

Our study started from the premise that the MBL gene and its alleles, MBL2D and MBL2H, may have some clinical relevance in combination with standard biological markers in the evaluation of adult patients with bronchial asthma. We wanted to analyse the effectiveness of these genetic markers both in the diagnosis of allergic bronchial asthma in children and in the evolution of the disease among the population under the age of 18, having predictive potential for determining exacerbation episodes of the disease.

. Bronchial asthma, being a chronic disease with possible severe evolution, even with correctly administered treatment, can significantly affect the quality of life of patients by limiting the body's physical capabilities. In addition to decreasing the patient`s quality of life, it also causes significant costs in terms of medication and therapeutic methods used in the palliative treatment of these patients in the advanced stages of the disease.

**Objectives of the study:**

- Evaluation of the clinical (severity of symptoms) and paraclinical (FEV1 variability, presence of eosinophilia) characteristics of the patients included in the study, given that the study has as subjects children diagnosed with allergic asthma;
- Evaluation of family history (such as parents who smoke or have bronchial asthma, toxic environment, etc.) and personal medical history (atopia, known allergies, etc.);
- Evaluation of the clinical applicability in the diagnosis of allergic bronchial asthma in children of determining the plasma level of MBL in accordance with known biological markers: increased levels of eosinophil granulocytes, increased levels of plasma IgE;

- Evaluation of the role of MBL gene expression and MBL2D and MBL2H alleles in the etiology of allergic bronchial asthma in children and in the occurrence of exacerbation episodes.

The standard biological biomarkers in the diagnosis of allergic bronchial asthma are the number of eosinophil granulocytes and the level of plasma IgE. These biomarkers often show elevated values in patients diagnosed with allergic bronchial asthma. However, current studies focus on the discovery of new diagnostic and prognostic biomarkers in asthma. The plasma concentration of MBL is considered a new factor with multiple possibilities both in the initial evaluation and in the follow-up of the evolution of patients with bronchial asthma.

The scientific results in the field have shown high levels of circulating MBL in both adults and children with bronchial asthma, although few papers report the association of MBL values with the severity of symptoms. However, there are studies, which suggest that the level of MBL could play a modulatory role in the progression of bronchial asthma, thus being a marker for the negative evolution of the disease.

This study aims to contribute to the development of a possible minimally invasive diagnostic and prognostic test, based on the measurement of the serum level of MBL in accordance with the plasma level of IgE and eosinophil cells in allergic bronchial asthma from a young age.

**Objectives of Study 1:**

- Evaluation of the clinical (severity of symptoms) and paraclinical (variability of FEV1, presence of eosinophilia) characteristics of the patients included in the study;
- Evaluation of family history and personal medical history;
- Evaluation of the clinical applicability in the diagnosis of allergic bronchial asthma in children of determining the plasma level of MBL in accordance with the known biological markers: the increase in eosinophil granulocytes and the increase in the plasma IgE level.

The study was carried out at the Paediatric Clinical Department II, Emergency County Clinical Hospital, Arad, Romania, over a period of 1 year between 2019 and 2020 and included 133 patients, of which 69 patients with asthma who attended a paediatric consultation for respiratory symptoms and 64 patients age-, sex-, and ethnicity-matched healthy subjects as a control group. From the study group, a number of 43 patients also agreed to the collection of laboratory samples.

We took the anamnesis of all patients to discover personal medical history, allergies, family history and consumption of toxic substances in the case of parents (alcohol, smoking, etc.).

In addition, ventilatory tests were performed to assess lung functions and blood samples were collected to determine the quantitative level of IgE and plasma concentrations of MBL.

Biostatistical methods helped us to analyse the data obtained.

In order to obtain as much information as possible, we performed a detailed anamnesis of the patients to know the aspects related to the felt symptoms, personal pathological antecedents (allergies and atopic conditions being favourable factors for the occurrence of allergic bronchial asthma), AHC (parents' toxic consumption, especially smoking), background and lifestyle.

The result of our study regarding the distribution of patients according to the environment of origin is consistent with international studies, which show a higher prevalence of bronchial asthma in urban areas.

Patients under 18 years of age known to have a history of allergic bronchial asthma, who presented for exacerbation of the disease and patients who presented the first episode of respiratory symptoms suggestive of this pathology, were included in the study.

In our study we included a total of 133 patients, of which 69 in the group of patients with bronchial asthma. We can observe the relatively homogeneous distribution between the sexes in all study groups. However, we can observe a slight predominance of the number of female patients diagnosed with bronchial asthma.

In our study, we found that 26.08% of asthmatic patients (n=69) presented family history of bronchial asthma. We can also observe that most children with bronchial asthma come from an environment where one or both parents smoke. Thus, in our study we observed that in 27.53% of cases only the mother smokes, in

36.23% of cases only the father smokes and in 14.49% of cases both parents smoke.

Our results show that there are no statistically significant differences in the evolution of the asthmatic disease in the patients included in the study, depending on the toxic consumption of the parents (Kruskal-Wallis test, p=0.438). Interestingly, the patient with the most severe evolution of the disease comes from a family of non-smokers.

However, if we calculate the average of the classes of drugs administered, we can see that patients with a smoking mother and those with both smoking parents needed an average of 3 different classes of treatment, while the other two categories of patients received chronic treatment constituted on average from 2 different drug classes.

In our study we followed both changes in auscultation, such as lung rales and expiratory wheezing, and changes in blood samples.

Our study investigated for the first time the potential clinical significance of the plasma MBL level in the context of asthma in children, these results being published in a specialist journal with an impact factor. Measured values showed wider heterogeneity in asthma patients than in controls.

Our results suggest that the plasma level of MBL compared to IgE values has a relative diagnostic role for asthma in children and adolescents, because MBL concentrations have a greater relevance in the diagnosis of asthma in children and greater sensitivity and specificity, considering found that all asthmatic patients had altered MBL values compared to IgE values, which were altered only in a few asthmatic patients. The discrepancy between IgE values within the study groups requires further study; further studies are needed to elucidate the role played by MBL in the determinism and evolution of asthma in minor patients.

Mannose-binding lectin (MBL) is the first component of complement in the lectin pathway and an acute phase reactant secreted by the liver. It is encoded in humans by a single functional gene (MBL2) located on chromosome 10q11.2 – q21. Low concentration and insufficiency of MBL are caused by polymorphisms in codons 52 (CGT → TGT; designated allele D), 54 (GGC → GAC; B), and 57 (GGA → GAA; C) in exon 1 of the MBL2 structural gene, resulting in Arg → Cys, Gly → Asp, and Gly → Glu amino acid substitutions in the peptide. The concentration of MBL is highly dependent on several polymorphisms of the promoter region of the MBL2

gene, of which the base pair (bp) at position -221 is clinically the most important (nucleotide change G → C; respectively Y → X alleles). The presence of the X allele in the homozygous state influences the basal serum MBL concentration equal to that single structural variant allele .

In Romania, due to the lack of studies, there are very few data related to the genetic determinism of bronchial asthma. International research has already shown that the MBL2 gene is an important factor of the innate immune system, and its poor functioning can cause increased susceptibility to infections, allergies and the development of bronchial asthma.

This study aims to add to the already known data about the MBL2 gene and its two important alleles. To the best of our knowledge, this study evaluates for the first time in Romania their importance in the etiopathogenesis of bronchial asthma, the severity and frequency of exacerbations of the disease in patients under 18 years of age.

**Objectives of Study 2:**

- Evaluation of the incidence of different genotypes;
- Evaluation of the incidence of different alleles;
- Assessment of exacerbations according to the allele present;
- Evaluation of the clinical applicability both in the diagnosis of allergic bronchial asthma in children, as well as the predictive value for the evolution of the disease.

This is a prospective study that aims to analyse the correlations between the MBL allele gene present and the number of exacerbations, respectively their severity in children and adolescents with bronchial asthma.

Study subjects are patients <18 years of age who met the inclusion criteria (children and adolescents, who showed variability in forced expiratory volume (FEV) and were under treatment with bronchodilators and antihistamines), whose parents gave written consent for participation. We also obtained informed consent from the adolescents.

The study was carried out at the Paediatric Clinical Section II, Arad County Emergency Clinic Hospital, Romania, over a period of 1 year between 2019 and

2020 and included 133 patients, of which 64 patients with bronchial asthma who participated in a consultation pediatric for respiratory symptoms and 69 age-, sex-, and ethnicity-matched healthy subjects as a control group.

Patients with the homozygous variant genotype were more than twice as likely to be ill compared to the other variants. Also, in homozygous patients the disease was diagnosed at younger ages compared to heterozygous ones.

The father's smoking status has been shown to be an important risk factor for the development of bronchial asthma in children from families with insignificant or absent heredity-collateral history of this disease.

As far as we know, this is the first study in Romania that examines the clinical relevance of the plasma level of MBL in the context of bronchial asthma in patients under 18 years of age. The results of our research should therefore extend the previous knowledge of the presumed link between MBL and the pathogenesis of asthma in children and provide new data on potential new tools to be used in simplifying and improving the diagnosis of this pathology in children and adolescents. .

Our study focused on the determination of plasma IgE levels in patients stratified on the basis of MBL and blood eosinophil levels in the systemic bloodstream. We found 12 asthma patients who had elevated levels of eosinophils in their blood samples, while control subjects had normal eosinophil counts. This suggests that the eosinophil count may be useful for delineating between children with asthma and healthy children. Once present in the airways and systemic blood circulation, eosinophils release some substances, which can cause changes in bronchoconstriction and epithelial cell damage. This damage, related to profibrotic cytokines also released by eosinophils and epithelial cells, can lead to airway remodeling .

Increased levels of MBL and its activity in allergic patients may contribute to additional complement activation through the lectin pathway and thus to increased severity of allergic markers such as increased blood eosinophils. Complementary activation of MBL can also lead directly to the development of allergies by affecting the level of proinflammatory cytokines.

Therefore, the study here aimed to contribute to the discovery of a possible minimally invasive diagnostic or prognostic test based on the measurement of serum

MBL levels in accordance with the level of IgE and eosinophil cells in allergic asthma from early ages.

In our study, plasma concentrations of MBL were significantly higher in children with asthma compared to healthy controls. We also found a positive correlation between MBL plasma concentrations and IgE levels in peripheral blood samples. These data provide evidence for an association between these two biomarkers, which may reflect similar molecular aspects underlying the disruption of airway functions. However, this association is difficult to consider to indicate a role of MBL in the pathogenesis of allergic asthma in children and adolescent patients, given the fact that this correlation does not imply causation. Rather, it should be considered an interesting finding that deserves further investigation.

Our study has its limitations. One such limitation is represented by the fact that the samples were collected during periods of remission in children already diagnosed with asthma. The second limitation is due to the fact that plasma levels of MBL can vary slightly between individuals depending on environmental factors (of for example, exposure to arsenic, lead, persistent organic pollutants, air pollution) and infections or other inflammatory pathologies, which could interfere with the results obtained.

However, our results are promising. We believe that determination of plasma MBL in the future can be used as a diagnostic and prognostic test in asthma. These findings support previous research studies that reported elevated levels of MBL in both adults and children diagnosed with asthma. Studies suggest that MBL could play a modulatory role in asthma progression and therefore be a susceptibility marker for severe disease or a marker for a distinct asthma phenotype. Two authors reported an association between MBL levels and eosinophilia, supporting our hypothesis of a role for MBL in different asthma phenotypes [40].

In our study we investigated the significance of different genotypes of MBL2, which may cause low concentration of MBL and insufficiency of MBL in children diagnosed with allergic asthma.

An important limitation of our study is represented by the lack of data on the role played by the MBL gene in the triggering of bronchial asthma, and in the rate of exacerbations in children. Compared to adults, children have a less developed immune system, so the course of the disease and the body's reactions to stimuli will be different.

When evaluating the effect of MBL2 genotype on the occurrence of atopy in controls, no statistically significant results were detected. However, when evaluating clinical symptoms among controls with atopy, lower respiratory tract symptoms tend to be less frequent in women carrying the A/O genotype than in those with the A/A genotype. Gender distribution differences in what regarding atopy and related disorders have been previously detected.

The opsonization defect caused by MBL deficiency has been observed to be associated with atopy in children [46]. However, the direct link between MBL insufficiency and atopy has not been previously studied.

Our results negate the previously suggested predisposing effect of MBL deficiency on atopy, at least in children. In fact, MBL deficiency may even provide protection against atopic symptoms in certain subgroups.

The originality of the doctoral thesis entitled "Predictive alleles of the risk of exacerbation in allergic bronchial asthma - MBL2D and MBL2H" lies in the fact that, to our knowledge, it is the first scientific paper in Romania, which traces the connection between the serum level of MBL and the risk of asthma exacerbation allergic bronchitis in children, respectively the alleles present in the patients included in the study, and their role in the evolution of the disease. The level of serum MBL proved to be an important marker in the diagnosis of allergic asthma in children with increased specificity and sensitivity compared to other markers. The remarkable discovery of this study is the fact that in the case of patients without hereditary-collateral history of bronchial asthma, tobacco consumption in the case of the father predisposed to the appearance of this disease. The results of this paper emphasize the importance of epigenetics in the case of allergic asthma in children, respectively the importance of the parents' lifestyle, which through tobacco consumption can cause the appearance of allergic asthma in children by modifying the epigenome, in the absence of hereditary-collateral antecedents for this disease. If genetics "gives birth" to life, epigenetics shapes it. This aspect brings into discussion the responsibility regarding the lifestyle of the parents in order not to determine the appearance of diseases in children. At the same time, knowing the genetic involvement in children with asthma can influence the approach and appropriate management of asthma in children to avoid the occurrence of severe forms of asthma in adulthood.