

## Original Paper

# Age-Dependent Prevalence of Persistent Wheezing Phenotypes in Romanian Children

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### REZUMAT

#### *Variația cu vârsta a prevalenței fenotipurilor de wheezing recurent la copiii din România*

Prezența atopiei este un element esențial în clasificarea wheezingului recurent pediatric. A fost descrisă o asocieră a fenotipurilor de wheezing recurent cu vârsta la copii; totuși, există diferențe de prevalență a fenotipurilor de wheezing recurent la copiii din regiuni diferit dezvoltate ale lumii. Am studiat relația cu vârsta a parametrilor imuni sanguini și a fenotipului de wheezing la 44 copii din România cu wheezing recurent sau astm, internați într-o perioadă de 24 luni într-un spital de copii, fie pentru investigații în stare clinică stabilă, fie pentru tratament în timpul reactivării patologiei respiratorii. Numărul de leucocite și cel de neutrofile sanguine au prezentat o creștere în primii 3 ani de viață, urmată de ușoară descreștere după aceasta, pe când numărul de eozinofile sanguine a prezentat o creștere continuă în prima decadă de viață în grupul studiat. Fenotipul de wheezing a prezentat o dependență similară cu vârsta: fenotipul de wheezing nonatopic a fost prezent la vârsta preșcolară, pe când fenotipul atopic a fost detectat la vârstă mai mare. Dependența cu vârsta a parametrilor imuni sanguini și a fenotipului wheezingului la copiii din România cu wheezing recurent sau astm se aseamănă cu aceea descrisă în cazul wheezingului persistent al copilului în regiuni dezvoltate ale lumii.

**Cuvinte cheie:** fenotip de wheezing recurent la copil, astm pediatric

### ABSTRACT

Presence of atopy is an essential element in the classification of childhood persistent wheezing. An association with specific periods of childhood has been described for wheezing phenotypes; however, differences in the prevalence of childhood wheezing phenotypes exist between differently developed regions of the world. We studied the relationship of blood immune parameters and wheezing phenotype with age in 44 Romanian children with recurrent wheezing or asthma admitted during a 24 months interval in a children's hospital either for investigations during stable respiratory state or for treatment during recurrence. White blood cells count and neutrophil count showed an increase in the first 3 years of age, followed by slight decrease after wards, while eosinophil count showed a continuous increase in the first decade of life in the studied group. The wheezing phenotype showed a similar age dependence: non-atopic wheezing was manifest during preschool years, while the atopic phenotype was detected at a higher age. The age dependence of blood immune parameters and wheezing phenotype in Romanian children with recurrent wheezing or asthma resemble those described for childhood persistent wheezing in developed regions of the world.

**Key words:** persistent wheezing phenotype in children, pediatric asthma

## INTRODUCTION

Asthma is a heterogeneous group of disease entities with common characteristics such as intermittent respiratory symptoms (wheezing, tightness, cough, dyspnea), reversible airway obstruction and bronchial hyperresponsiveness [1]. Wheezing, caused by airflow restriction through narrowed airways, is the main clinical sign associated with asthma.

Two concepts related to asthma are currently in a process of disentanglement: the concept of asthma phenotypes (particular clinical characteristics, without reference to the underlying pathologic process) and endotypes (pathological mechanisms of disease) [2]. The disentangling of asthma phenotypes and endotypes is important for the prediction of disease evolution and for accurate therapeutic action.

Classifications of pediatric wheezing have been proposed based on clinical criteria related to breathing difficulty alone, such as the Tucson Children's Respiratory Study (TCRS) [3] or the Avon Longitudinal Study of Parents and Children (ALSPAC) [4].

A pediatric wheezing classification that takes into account both clinical aspects and immune endotype identified three phenotypes: transient wheezing in infancy, non-atopic persistent wheezing and immunoglobulin E-associated/atopic persistent wheezing [5,6].

Transient early wheezing typically begins in the first year of life and resolves up to 3-5 years of age, is not commonly associated with family history of asthma or with atopy [7] but with mechanical pulmonary particularities [8].

Non-atopic persistent wheezing has its first episode in the first year of life [9], shows decreasing frequency of episodes by early adolescence [10] and is not associated with allergic sensitisation [6]. Lower respiratory infections of viral etiology, particularly with respiratory syncytial virus (RSV) are the most prevalent cause of non-atopic wheezing in the first decade of life. RSV infection before the age of 3 years was associated with increased risk of wheezing during the first decade of life, independently of other risk factors for asthma such as family history of asthma or atopy; it was not associated with either increased risk of allergic sensitisation or higher total serum IgE levels; the risk of asthma development after RSV lower respiratory infection decreased with age, being insignificant by 13 years of age [10].

The IgE-associated/atopic persistent wheezing develops after the first year of life and persists into later adolescence [3]. It is associated with atopy and increased airway hyper-responsiveness, male gender [3], early sensitisation to food or aeroallergens [11,12] and the development of symptoms between wheezing exacerbations [13].

Asthma classifications based on immune parameters in adults make use of procedures such as sputum induc-

tion that are difficult to perform in children due to both ethical and practical reasons. Asthma classifications in children use easier to measure parameters in the blood such as those that define atopy. Recent studies show that blood eosinophil count may be used as a good predictor of the airway eosinophil level in subjects with asthma [14,15].

The wheezing or asthma phenotype depends on gender, genetic background, age and environmental exposures [5]. The age dependence of immune endotype in children is a subject of great interest due to several reasons. Firstly, the immune endotype may change in an individual during childhood years: a study of induced sputum inflammation in children, similarly to the investigation in adults, showed that 41% of children with asthma fulfilled on different occasions the induced sputum criteria for eosinophil or non-eosinophil asthma without changes in treatment or measured exhaled NO [16]. Secondly, a difference in asthma phenotype prevalence in children has been described between differently developed world regions (developed regions have a higher prevalence of the persistent atopic phenotype, while underdeveloped regions show a higher prevalence of the persistent non-atopic phenotype) [6,17] and between urban and rural areas in the same country [18].

Considering the lack of data regarding the phenotype prevalence in Romanian children, we proposed to investigate the age dependence of both blood immune parameters and wheezing phenotype in Romanian children with recurrent wheezing and asthma.

## MATERIALS AND METHODS

### Study sample

We performed a retrospective study on children diagnosed with recurrent wheezing or asthma that were admitted either for recurrence or for routine checkup in "Dr. V. Gomoiu" Children's Hospital, Bucharest during a 24 months interval (2013-2014). Recurrent wheezing or asthma diagnosis was established by a pediatric pneumologist based on clinical and lung function criteria. Subjects with simultaneous non-respiratory illness or with chronic respiratory illness non-related to asthma were excluded. Clinical state was considered stable if subjects had shown no respiratory infection, wheezing or asthma episode or medication change in the 4 weeks prior to hospital admission. The persistent wheezing phenotype (atopic vs. non-atopic) was established by the pediatric pneumologist based on the presence of clinical signs of atopy, positive results of skin prick tests and increased IgE levels to specific allergens in the subjects' medical history.

The study was done in accordance with the subject protection provisions of "Carol Davila" University of Medicine and Pharmacy and "Dr. V. Gomoiu" Children's Hospital Bucharest.

### Study parameters

Studied parameters were gender, age, complete blood count, eosinophil/lymphocyte ratio (ELR), eosinophil/neutrophil ratio (ENR) and neutrophil/lymphocyte ratio (NLR), respiratory state (stable vs. recurrence), maintenance inhaled corticosteroid treatment and persistent wheezing phenotype (atopic vs. non-atopic). Data were retrospectively collected from patient files. Measurements were routinely performed using automated clinical biochemistry systems on venous blood collected within 24 hours from admission to the hospital.

### Statistical analysis

Statistical analysis was performed using the R statistical language software, ver. 3.2.0 [19]. The non-parametric Wilcoxon rank sum test was used to study differences in continuous variables with respect to binomial categorical variables. Pearson's chi-squared test was used to analyse the association of categorical variables.

The Davies' test applied to linear regression was used for trend change point detection. Linear regression with segmented relationships was used for slopes detection around trend change points. Necessary methods were available in the "segmented" library of the R language [20].

p values between 0.05 and 0.1 were considered suggestive (+), between 0.01 and 0.05- significant (\*) and under 0.01- highly significant (\*\*).

Data were shown as median, first and third quartiles where appropriate.

## RESULTS

### Structure of the study sample

44 subjects aged 1.2 to 16.3 years were studied, of which 29 were boys and 15 girls. The median age was 7.1

years, the first and third quartiles were: 4.7 and 11.1 years in the study group.

Age, maintenance corticosteroid use, phenotype (atopic vs. non-atopic), respiratory state (stable vs. recurrence) and immune parameters were balanced with respect to gender (data not shown), with the exception of eosinophil count which showed significant gender-related difference (boys had higher eosinophil blood count than girls): median, [first and third quartiles] for eosinophil count were 0.42, [0.19, 0.66]  $\times 10^3/\mu\text{l}$  for boys and 0.17, [0.10, 0.32]  $\times 10^3/\mu\text{l}$  for girls, Wilcoxon rank sum test  $p=0.02$  (\*\*).

### Age dependence of blood immune parameters

Blood immune parameters showed age-dependent variation in the study group (Fig. 1). Trend change points were detected in the white blood cells count and neutrophil count at approximately 3 years of age and in eosinophil count, ELR and ENR at approximately 11 years of age (increase before the trend change point followed by decrease afterwards).

### Age dependence of subjects' persistent wheezing phenotype

Persistent wheezing phenotype (atopic vs. non-atopic) was significantly ( $p<0.05$ ) associated with respiratory state (non-atopic wheezers showed more frequent recurrence) and suggestively ( $p<0.1$ ) associated with inhaled corticosteroid use (atopic wheezers more frequently used inhaled corticosteroids). A highly significant difference ( $p<0.01$ ) was detected in the age of subjects with respect to the persistent wheezing phenotype (atopic wheezing was associated with higher age) (Table 2, Fig. 2).

Extremely significant differences ( $p<0.0001$ ) were found in eosinophil count, ELR and ENR with respect to the persistent wheezing phenotype (higher eosinophil count and ratios were associated with atopic wheezing) (Table 2).

Table 1. Trend change of immune parameters in relation with age

| Parameter               | p          | Trend change age (years mean $\pm$ SE) | Trend slopes (mean $\pm$ SE)         |
|-------------------------|------------|--|--------------------------------------|
| White blood cells count | 0.01 *     | 2.84 $\pm$ 0.75                        | 4.42 $\pm$ 3.02<br>-0.59 $\pm$ 0.12  |
| Lymphocyte count        | 0.75       |  |                                      |
| Monocyte count          | 0.16       |  |                                      |
| Neutrophil count        | 0.02 *     | 2.89 $\pm$ 0.83                        | 3.73 $\pm$ 2.67<br>-0.46 $\pm$ 0.1   |
| Basophil count          | 0.75       |  |                                      |
| Eosinophil count        | <0.0001 ** | 10.88 $\pm$ 0.81                       | 0.06 $\pm$ 0.01<br>-0.12 $\pm$ 0.03  |
| ELR                     | <0.0001 ** | 11.09 $\pm$ 0.79                       | 0.02 $\pm$ 0.005<br>-0.03 $\pm$ 0.01 |
| ENR                     | <0.0001 ** | 11.14 $\pm$ 0.92                       | 0.03 $\pm$ 0.005<br>-0.05 $\pm$ 0.01 |
| NLR                     | 0.38       |  |                                      |

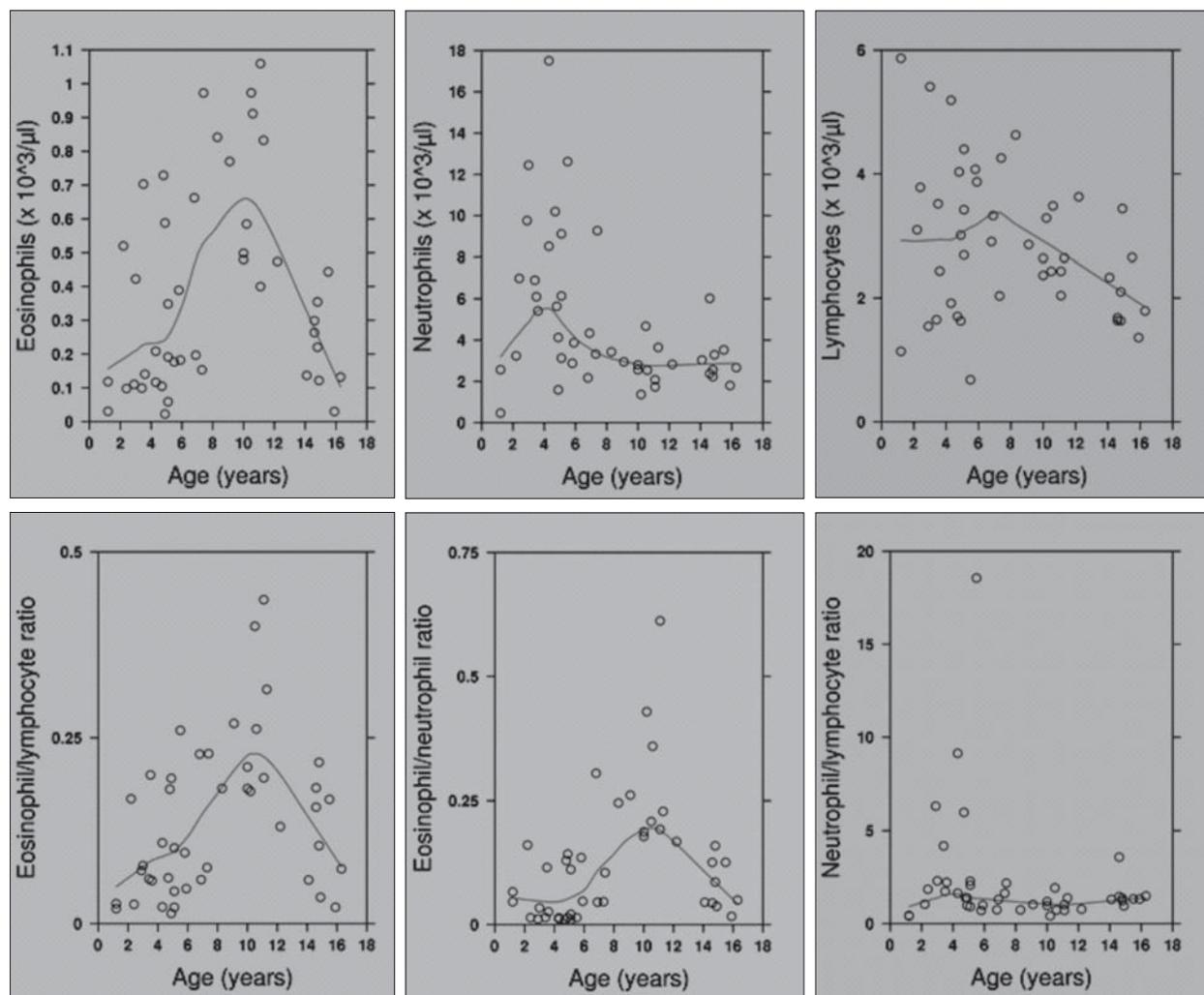
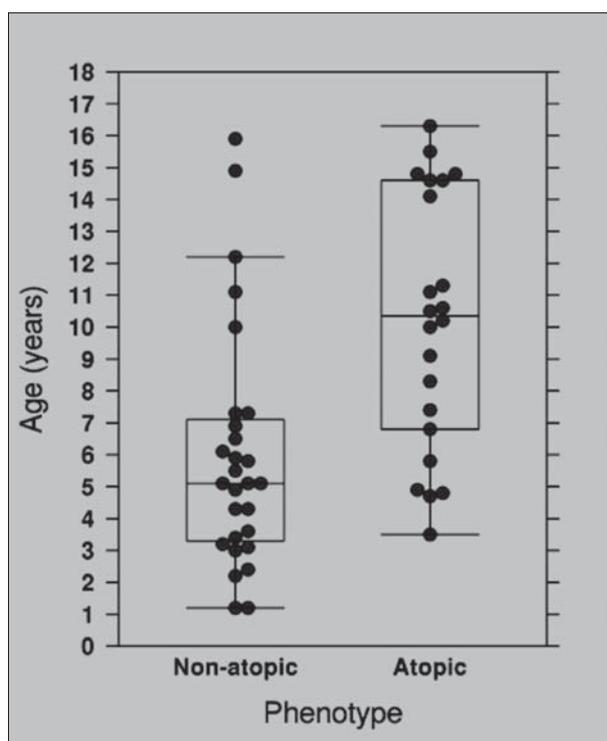


Figure 1. Age dependence of subjects' immune parameters

Table 2. Dependence of subjects' parameters on the persistent wheezing phenotype

| Parameter                                      | Persistent wheezing phenotype |                   |              |
|--|-------------------------------|-------------------|--------------|
|  | Non-atopic                    | Atopic            | p            |
| Count  | 22                            | 22                | -            |
| Age (years)                                    | 5.1 (3.4; 7.2)                | 10.3 (6.9; 14.4)  | 0.003 (**)   |
| Gender (% male)                                | 59%                           | 72%               | 0.52         |
| Inhaled corticosteroid use (% users)           | 36%                           | 68%               | 0.07 (+)     |
| Respiratory state (% recurrence)               | 72%                           | 36%               | 0.03 (*)     |
| White blood cells count (x10 <sup>3</sup> /μl) | 8.61 (7.13; 11.63)            | 7.51 (5.89; 9.76) | 0.28         |
| Lymphocytes (x10 <sup>3</sup> /μl)             | 2.89 (1.94; 3.74)             | 2.64 (2.15; 3.43) | 0.80         |
| Monocytes (x10 <sup>3</sup> /μl)               | 0.72 (0.53; 1.21)             | 0.66 (0.50; 0.89) | 0.55         |
| Neutrophils (x10 <sup>3</sup> /μl)             | 3.59 (2.63; 6.96)             | 2.99 (2.54; 4.53) | 0.26         |
| Basophils (x10 <sup>3</sup> /μl)               | 0.02 (0.01; 0.09)             | 0.02 (0.01; 0.03) | 0.38         |
| Eosinophils (x10 <sup>3</sup> /μl)             | 0.16 (0.10; 0.31)             | 0.58 (0.31; 0.81) | <0.0001 (**) |
| Eosinophil/lymphocyte ratio                    | 0.05 (0.02; 0.10)             | 0.18 (0.15; 0.22) | <0.0001 (**) |
| Eosinophil/neutrophil ratio                    | 0.03 (0.01; 0.06)             | 0.13 (0.10; 0.24) | 0.0001 (**)  |
| Neutrophil/lymphocyte ratio                    | 1.31 (0.97; 2.18)             | 1.34 (0.81; 1.47) | 0.58         |



**Figure 2.** A difference in subject age is associated with wheezing phenotype. Wilcoxon rank sum test  $p=0.003$  (\*\*)

## DISCUSSION

In the present work we report the results we obtained in the investigation of age dependence of blood immune parameters and wheezing phenotype in a group of Romanian children with recurrent wheezing or asthma.

The study sample consisted of 44 subjects aged 1.2 to 16.3 years (29 boys and 15 girls) that were admitted during a 24 months period (2013-2014) to “Dr. V. Gomoiu” Children’s Hospital, Bucharest for recurrence or for investigations during stable clinical state.

The subjects’ immune parameters and phenotype were balanced with respect to gender. Blood eosinophils alone showed significant differences in this regard (boys had significantly higher eosinophil counts than girls). This result is in accordance with published data that showed higher prevalence of atopy in boys than in girls before puberty [21].

Of the subjects’ immune parameters, white blood cell counts, eosinophil and neutrophil counts, eosinophil/lymphocyte and eosinophil/neutrophil ratios showed significantly discontinuous trend variation at specific age values, with increase before the trend change point and decrease afterwards: the trend change points for neutrophil counts was detected at approximately 3 years of age, while for eosinophil count at approximately 11 years of age.

The association of wheezing phenotype with blood eosinophil count, ELR and ENR is not surprising.

Although corticosteroid use is known to promote eosinophil apoptosis and neutrophil survival [22,23], we haven’t detected significant differences in blood eosinophil and neutrophil counts or their ratios with respect to inhaled corticosteroid use, which is in accordance with other investigators’ findings [24]. Inhaled corticosteroid use, while most probably significantly modifying airway inflammation, did not influence blood eosinophil and neutrophil counts and their ratios.

Wheezing phenotype showed a highly significant association with age (atopic wheezing was associated with higher age). This finding is similar to the age distribution of persistent wheezing phenotypes reported in developed areas of the world, with non-atopic wheezing being prevalent during preschool years and the atopic phenotype at a higher age [6].

A significant association was detected between wheezing phenotype and inhaled corticosteroids use (atopic wheezers more frequently used inhaled corticosteroids). This is in accordance with the known effects of corticosteroids of promoting eosinophil apoptosis [22] and with the findings of higher corticosteroid sensitivity of atopic compared to non-atopic asthma [25-29].

A suggestive association was also found between the respiratory state (stable vs. recurrence) and the wheezing phenotype: children with the non-atopic phenotype more frequently showed recurrence. This is in accordance with our data on the significant association of both phenotype and respiratory state with age (recurrence, like non-atopic phenotype, was significantly associated with small age) and with eosinophil/neutrophil balance (recurrence, like non-atopic wheezing, was significantly associated with decreased eosinophil/neutrophil blood ratio) (data in press).

The obtained results show an age-dependent prevalence of wheezing phenotypes in Romanian children that resembles the one described in developed countries: increased prevalence of non-atopic asthma in preschool years and of atopic asthma at higher age [6]. The obtained results further show that blood eosinophil and neutrophil counts and their ratios, while not associated with inhaled corticosteroids use, paralleled the age distribution of the wheezing phenotypes: neutrophil count increased in early childhood, followed by slight decrease, while eosinophil count continuously increased during the first decade of life. The observed age-dependent distribution of wheezing phenotype and immune parameters has important consequences for the prediction of the subjects’ response to treatment.

To our knowledge, there is no previously published data on the age-dependence of immune parameters and phenotype in Romanian children with recurrent wheezing or asthma. Our study is the first to investigate the age dependence of the wheezing phenotype in Romanian children. The studied children are inhabitants of the south-eastern region of the country. Further investigation

is necessary in order to evaluate the asthma characteristics of children in other regions of the country and the relation of wheezing phenotype with the type of living environment (urban vs. rural).

Deficiencies of our study stem from the relatively low number of studied subjects (inherent to the choice of a single study site), from the cross-sectional nature of the study (the age-dependent prevalence of immune parameters and wheezing phenotype was not studied longitudinally in the individual subjects) and from the retrospective character of the study (dependence on medical records).

In conclusion, the obtained results suggest that both immune parameters and persistent wheezing phenotype have an age-dependent prevalence in Romanian children that resembles the age distribution described in developed areas of the world.

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