ORIGINAL PAPERS

Vitamin D Levels Correlate with Wheezing and Asthma Recurrence in Children

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Abstract

Objective: Vitamin D has pleiotropic actions, including a role in the development of the immune response. A link was suggested between vitamin D deficiency and the development of asthma in children. The aim of the present study was to investigate the role of serum 25(OH) vitamin D level as a predictor of recurrent wheezing and asthma exacerbations in children. **Material and method:** We investigated the parameters of 52 children aged 0.8 to 16.3 years, diagnosed with recurrent wheezing or asthma who were admitted to a children's hospital during a 24 months period either for recurrence or during clinically stable state. **Results:** Of the investigated parameters, eosinophil count and eosinophil/lymphocyte ratio were significantly associated ($p<0.05$) with serum 25(OH)D levels. Clinical state (stable/recurrence) was suggestively associated ($p\leq0.1$) with several individual parameters: serum 25(OH) D level, age, neutrophil and eosinophil counts and their ratios (eosinophil/neutrophil and neutrophil/lymphocyte ratio). However, in binomial regression analysis serum 25(OH) D level was the only significant ($p<0.05$) independent predictor of the clinical state. **Conclusion:** Serum vitamin D level was found to be related to clinical state (stable/recurrence) in children with recurrent wheezing or asthma.

Keywords: recurrent wheezing, asthma, vitamin D, pediatrics

Rezumat

Obiectiv: Vitamina D are efecte multiple, inclusiv un rol în dezvoltarea sistemului imun. A fost sugerată o legătură între deficitul de vitamina D și dezvoltarea astmului bronșic la copii. Scopul prezentului studiu a fost să investigheze rolul nivelului seric al 25(OH) vitaminei D ca predictor al exacerbărilor de wheezing recurent și de astm bronșic la copii. **Material și metodă:** Am analizat parametrii a 52 de copii cu vârsta între 0,8 și 16,3 ani, diagnosticați cu wheezing recurent sau astm bronșic, care au fost internați într-un spital de copii într-o perioadă de 24 de luni, fie pentru recurență, fie în stare clinică stabilă. **Rezultate:** Dintre parametrii investigați, numărul de eozinofile și raportul eozinofile/limfocite au fost asociați semnificativ ($p<0,05$) cu nivelurile serice ale 25(OH)D. Statusul clinic (stabil/recurență) a fost asociat sugestiv ($p\leq0,1$) cu mai multi parametri individuali: nivelul seric al 25(OH)D, vârsta, numărul de neutrofile și de eozinofile și raporturile lor (raportul eozinofile/neutrofile și neutrofile/limfocite). Totuși, în analiza prin regresie binomială nivelul seric al 25(OH)D a fost singurul predictor independent semnificativ ($p<0,05$) al statusului clinic. **Concluzie:** Există o relație între nivelul seric al vitaminei D și statusul clinic (stabil/recurență) la copiii cu wheezing recurent sau astm bronșic.

Cuvinte cheie: wheezing recurent, astm bronșic, vitamina D, pediatrie

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INTRODUCTION

Vitamin D is a seco-steroid with a pivotal role in the calcium and phosphate metabolism, two ions that are essential for bone mineralization, neuromuscular activity and general cell functions.

Sources of vitamin D are either endogenous (production in the skin from 7-dehydrocholesterol under the influence of sunlight UV-B radiation)[1] or exogenous (dietary). The dietary forms of vitamin D are either of vegetal origin (ergocalciferol, vitamin D2)[2] or of animal origin (colecalciferol, vitamin D3)[3]. The two calciferol forms are similarly metabolized in humans, have equal bioavailability and effectiveness[4].

Vitamin D and its products are transported in the body through the blood, being bound by vitamin D-binding protein (DBP)[5].

In humans vitamin D suffers two successive hydroxylation steps, at positions 25 and 1, in order to generate the metabolically active vitamin D product. The hydroxylation at position 25 takes place in the liver[6], being catalysed by cytochrome P450 enzymes such as CYP27A1- and CYP2R1- hydroxylases[7].

Hydroxylation at position 1 is catalysed by the cytochrome P450 enzyme 25-hydroxyvitamin D3 1alpha-hydroxylase (CYP27B1)[8,9]. An important amount of $1,25(OH)_2D$ is produced in the kidney[10]; however, the 1alpha-hydroxylase is also expressed at other sites, catalysing the local production of $1,25(OH)_2D$: in the skin[11,12], bone[13], immune cells[14].

Similarly to steroid hormones, $1,25(OH)_2D$ crosses the cellular plasma membrane and interacts with a specific nuclear receptor, the vitamin D receptor (VDR)[15]. $1,25(OH)_2D$ is considered the main activator of VDR since its affinity for the receptor is 1000-fold higher when compared to that of 25(OH)D[16].

Since $1,25(OH)_2D$ is produced locally, the vitamin D load in the body is given by the serum level of 25(OH)D.

Vitamin D exerts its actions through gene expression induction by binding, together with the vitamin D receptor (VDR) and transcription factors, to specific vitamin D responsive elements (VDREs) located in gene promoters[17].

The role of vitamin D in the calcium and phosphate metabolism is one of enhancement of intestinal reabsorption and of bone resorption of both ions, with the purpose of maintaining their constant levels in the blood[18].

A role of vitamin D in other physiologic mechanisms is suggested by the expression of VDR in tissues not involved in calcium and phosphate metabolism[19]. Vitamin D regulates up to 5% of the human genome, inducing physiologic responses in at least 36 cell types[19,20]. White cells stimulated in vivo with vitamin D modify the expresion of hundreds of genes, predominantly involved in immunological responses[21].

Vitamin D acts on the immune cells in several ways: endocrine, paracrine, autocrine[22,23].

Vitamin D has immunomodulatory effects on both innate and adaptive immune systems[24]. It was shown to favour an anti-inflammatory Th2 type immune response in the detriment of the pro-inflammatory Th1 or Th17 types, through several mechanisms: by decreasing the Th1 cell counts[25] and Th17 cell counts[26,27], by direct effect on the differentiation of naïve T cells into Th2 cells[28], by increasing the synthesis of Th2 cytokines[29,30] and decreasing the synthesis of Th1 cytokines[31,32] and of Th17 cytokines[33].

Reduced vitamin D levels were found in diseases with increased Th1 response, such as rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis[34].

The role of the deficit of vitamin D in asthma is to be clarified, since asthma is generally considered to be associated with an increased Th2 activation, while a deficit of vitamin D would favor the balance switch in the opposite direction, towards Th1 or Th17 activation. A correlation of vitamin D levels with asthma has been frequently described in children[35,36], but seldom in adults[37-40]. In asthmatic children the vitamin D deficit (serum concentrations under 30 ng/ml) was associated with an increased risk of hospitalization, with severe exacerbations and with an increased risk of poor control of the disease[35,41-43].

The aim of the current study was to evaluate serum vitamin D as a predictor of recurrence of wheezing or asthma in children.

MATERIAL AND METHOD

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 to "Victor 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 not 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 preceding hospital admission.

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

Study parameters

Studied parameters were age, gender, respiratory state (stable/recurrence), maintenance corticosteroid use, month of birth and month of blood collection, complete blood count, blood 25(OH)D, calcium, magnesium, phosphate, iron and plasma proteins concentrations.

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.

25-hydroxy vitamin D (25[OH]D) concentrations were measured using ELISA assays (Euroimmun, Germany). Serum vitamin D levels were considered deficient for concentrations under 12 ng/ml, insufficient for concentrations between 12 and 20 ng/ml and normal for concentrations over 20 ng/ml[44].

Statistical analysis

Statistical analysis was performed using the R statistical language software, ver. 3.2.0[45].

The non-parametric Wilcoxon rank sum test was used for comparisons between two groups of continuous data. Pearson's chi-squared test was used for the investigation of categorical variables association. Linear regression and binomial regression analysis were used for investigating the quantitative contribution of independent determinants of a study parameter.

p values between 0.05 and 0.1 were considered "suggestive", between 0.01 and 0.05 were considered "significant" and under 0.01- "highly significant". The Hosmer-Lemeshow test for goodness of fit of logistic regression models has a null hypothesis that the model is fit; in this test a high p value ($p>0.05$) is used to show that the hypothesis of the model being fit cannot be rejected.

Data in tables and in the figure are shown as median, first quartile and third quartile where appropriate.

RESULTS

Structure of the study sample

52 subjects aged 0.8 to 16.3 years were studied, of which 35 were boys and 17 girls. The study sample was balanced with respect to subject gender. Of all parameters of the complete blood count, eosinophil count showed significant gender-related difference (boys had higher eosinophil count than girls) (data not shown). Serum vitamin D concentrations in the studied sample were (median, first and third quartile): 26.50 (16.80; 32.70) ng/ml.

Determinants of vitamin D concentration in the studied subjects

We studied the differences induced in subjects' clinical parameters by serum vitamin D concentration taken as a categorical variable with two levels ("sufficient": ≥20 ng/ml, "not sufficient": <20 ng/ml) in accordance with currently accepted values[44]. The eosinophil count and the eosinophil/lymphocyte ratio showed significant differences, while gender, clinical state, month of measurement and serum phosphate level were only suggestively associated with serum vitamin D concentration (Table 1). Lower vitamin D concentration was associated with female gender, recurrence of wheezing or asthma, lower eosinophil count and lower eosinophil/lymphocyte ratio (Table 1).

Maintenance inhaled corticosteroid use was not significantly associated with vitamin D levels (Table 1).

The association between serum vitamin D concentration and month of measurement shows seasonal aspect (Figure 1).

We studied in both linear and binomial regression tests the quantitative prediction of serum vitamin D concentrations by subjects' clinical parameters that showed individual association (gender, clinical state, month of measurement, eosinophil count, eosinophil/lymphocyte ratio and serum phosphate level). Linear regression analysis did not generate a significant model, while binomial regression analysis did not converge on a model (data not shown).

Determinants of recurrent wheezing or asthma state (stable/recurrence)

Age, neutrophil count, eosinophil/neutrophil ratio and neutrophil/lymphocyte ratio showed significant differences with respect to the clinical state, while white blood cells count, eosinophil count, hemoglobin concentration and serum 25(OH)D concentration showed only suggestive differences with respect to the clinical state (Table 2). Recurrence was associated with smaller age, lower serum 25(OH)D concentration, lower eosinophil count and lower eosinophil/neutrophil ratio, higher white blood cells count and neutrophil count, higher neutrophil/lymphocyte ratio and lower hemoglobin concentration (Table 2). Clinical state was not associated with month of measurement (data not

Table 1. Relationship between subjects' parameters and serum vitamin D levels

Parameter	Serum 25(OH)D concentration		*p*
	Sufficient (≥20 ng/ml)	**Not sufficient (<20 ng/ml)**	
Count	32	20	-
Age (years)	6.3 (4.87; 9.32)	7.55 (3.30; 14.22)	0.94
Gender (% male)	78.12	50	**0.07 (+)**
Month of birth (subjects per month)	2,2,2,0,2,3,4,2,3,6,2,4	1,4,1,3,3,1,1,1,1,1,2,1	0.37
Month of measurement (subjects per month)	0,4,7,1,0,0,5,2,8,4,1,0	0,1,3,5,0,0,0,0,5,3,2,1	**0.1 (+)**
Clinical state (% stable)	56.25	25	**0.05 (+)**
Maintenance inhalatory corticosteroid use (% use)	43.75	45	1
White blood cells (x10^3/µl)	8.32 (6.47; 11.05)	7.64 (5.89; 9.98)	0.52
Red blood cells (x10^6/µl)	4.83 (4.63; 4.94)	4.85 (4.6; 5.02)	0.98
Platelets (x10^3/µl)	292 (259; 364)	288 (220.5; 396.5)	0.96
Lymphocytes (x10^3/µl)	2.75 (2.03; 3.75)	2.65 (1.74; 3.49)	0.91
Monocytes (x10^3/µl)	0.71 (0.53; 1.04)	0.70 (0.46; 0.93)	0.41
Neutrophils (x10^3/µl)	3.53 (2.82; 5.58)	3.03 (2.56; 6.11)	0.51
Basophils (x10^3/µl)	0.02 (0.01; 0.04)	0.02 (0.01; 0.05)	0.71
Eosinophils (x10^3/µl)	0.37 (0.18; 0.71)	0.13 (0.1; 0.45)	**0.03 (*)**
Eosinophil / lymphocyte ratio, ELR	0.18 (0.07; 0.22)	0.07 (0.03; 0.16)	**0.02 (*)**
Eosinophil / neutrophil ratio, ENR	0.12 (0.03; 0.18)	0.04 (0.02; 0.12)	0.19
Neutrophil / lymphocyte ratio, NLR	1.36 (1.02; 1.85)	1.3 (0.86; 2.06)	0.59
Total serum calcium (mg/dl)	9.9 (9.4; 10.3)	9.4 (9.2; 10.3)	0.44
Ionized serum calcium (mg/dl)	4.4 (4.1; 4.5)	4.2 (3.95; 4.35)	0.30
Serum magnesium (mg/dl)	2.3 (2.3; 2.4)	2.5 (2.5; 2.5)	0.37
Serum phosphate (mg/dl)	5.2 (4.87; 5.57)	4.2 (4; 4.8)	**0.05 (+)**
Plasma proteins (g/dl)	7.2 (6.97; 7.55)	7.3 (6.75; 7.7)	0.96
Serum iron (µg/dl)	60.5 (51.5; 85)	91 (54; 100.2)	0.19
Hemoglobin (g/dl)	13.36 (12.75; 14.18)	13.17 (12.76; 14.02)	0.70
Haematocrit (%)	40.54 (38.97; 42.31)	40.61 (38.02; 42.08)	0.61

shown). Maintenance inhaled corticosteroid use was not significantly associated with respiratory state (Table 2).

After identifying age, vitamin D concentration, white blood cells, eosinophil and neutrophil counts, eosinophil/neutrophil and neutrophil/lymphocyte ratios

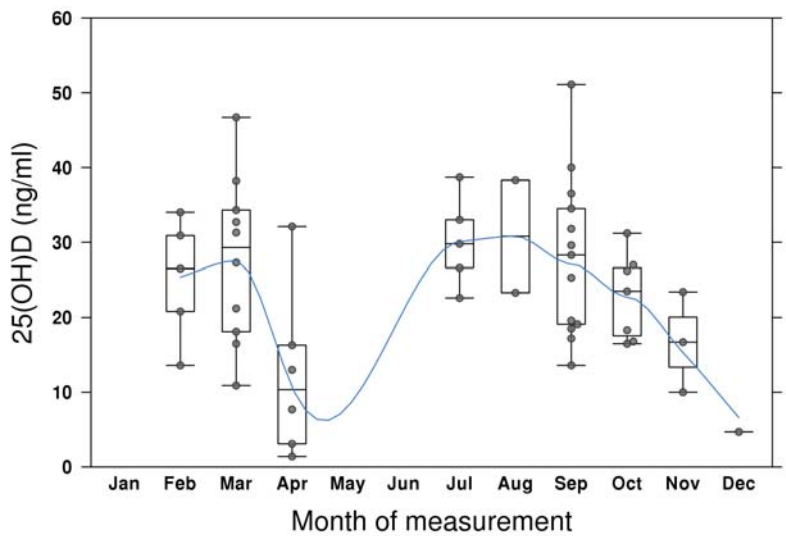

Figure 1. Dependence of serum 25(OH)D concentration on the month of measurement.
Serum 25(OH)D concentration was taken as a continuous variable. p=0.04 (*)

Table 2. Relationship between subjects' clinical parameters and the recurrent wheezing or asthma state (stable/ recurrence)

Parameter	Respiratory state		
	Stable	Recurrence	p
Count	23	29	-
Age (years)	**9.1 (5.85; 12.95)**	**4.9 (3.4; 7.4)**	**0.01 (*)**
Gender (% male)	78.26	58.62	0.15
Maintenance inhalatory corticosteroid use (% use)	47.82	41.37	0.78
Serum 25(OH)D conc. (ng/ml)	**29.6 (21.9; 32.4)**	**19.6 (16.5; 28.3)**	**0.1 (+)**
White blood cells (x 10^3/µl)	**7.62 (5.73; 8.76)**	**9.02 (6.97; 13.11)**	**0.07 (+)**
Red blood cells (x 10^6/µl)	4.85 (4.62; 4.98)	4.82 (4.67; 4.98)	0.90
Platelet count (x 10^3/µl)	286 (247.5; 342.5)	292 (225; 376)	0.78
Lymphocyte count (x 10^3/µl)	2.88 (2.3; 3.54)	2.63 (1.79; 3.51)	0.48
Monocyte count (x 10^3/µl)	0.59 (0.49; 0.78)	0.76 (0.59; 1.16)	0.19
Neutrophil count (x 10^3/µl)	**2.91 (2.34; 3.70)**	**5.41 (2.82; 8.51)**	**0.01 (*)**
Basophil count (x 10^3/µl)	0.02 (0.01; 0.03)	0.02 (0.01; 0.05)	0.56
Eosinophil count (x 10^3/µl)	**0.37 (0.21; 0.68)**	**0.17 (0.11; 0.47)**	**0.05 (+)**
Eosinophil / lymphocyte ratio, ELR	0.17 (0.08; 0.21)	0.07 (0.05; 0.18)	0.11
Eosinophil / neutrophil ratio, ENR	**0.13 (0.04; 0.23)**	**0.04 (0.01; 0.12)**	**<0.01 (**)**
Neutrophil / lymphocyte ratio, NLR	**1.1 (0.74; 1.37)**	**1.63 (1.04; 2.28)**	**0.01 (*)**
Total serum calcium (mg/dl)	9.7 (9.27; 10.15)	9.4 (9.15; 10.3)	0.96
Ionized serum calcium (mg/dl)	4.25 (4.12; 4.4)	4.25 (3.92; 4.4)	0.64
Serum magnesium (mg/dl)	2.4 (2.27; 2.5)	2.4 (2.3; 2.5)	0.84
Serum phosphate (mg/dl)	5.2 (4.87; 5.42)	4.3 (4.2; 4.9)	0.18
Plasma proteins (g/dl)	7.3 (6.8; 7.52)	7.2 (7.05; 7.75)	0.32
Serum iron (µg/dl)	68 (54; 90.5)	71 (50; 98)	0.75
Hemoglobin (g/dl)	**13.62 (12.97; 14.34)**	**13.02 (12.54; 13.46)**	**0.06 (+)**
Haematocrit (%)	40.99 (39.95; 42.25)	39.72 (37.95; 41.66)	0.15

and hemoglobin concentration as individual parameters that showed significant or suggestive differences in relation with subjects' clinical state, we investigated by binomial regression analysis their quantitative contribution as independent determinants of the clinical state (Table 3). Serum vitamin D concentration was a significant determinant, while age, white blood cells count and neutrophil count were only suggestive determinants of clinical state in binomial regression analysis (Table 3). The R^2 statistic on the obtained binomial regression model showed an important amount of variance to be explained by the predictors in the model. In the Hosmer-Lemeshow test of goodness of fit, due to the way the null hypothesis is formulated, higher p values ($p>0.05$) show the model to be fit, with greater significance for values closer to 1. Our model showed good fitness for the experimental data.

DISCUSSION

In the present study we have investigated the role of serum vitamin D as a predictor of wheezing and asthma recurrence in children. Our initial hypothesis that decreased vitamin D serum concentration associates with wheezing or asthma recurrence was confirmed by the study results.

Our study was performed retrospectively on a sample consisting of 52 Romanian children aged between 0.8 and 16.3 years diagnosed with recurrent wheezing or asthma who were admitted in a 24 months interval to an university hospital either during recurrence or for medical investigations in a stable respiratory state.

The study group was balanced with respect to gender. Among the investigated immune parameters blood eosinophil count showed significant gender-related difference. This finding is in accordance with previous studies in children that showed higher prevalence of asthma and atopy in boys than in girls before puberty[46].

The threshold for differentiating "sufficient" vs. "non-sufficient" serum 25(OH)D levels in children is a matter of debate. Some authors recommend the thresholds of 30 ng/ml (72.5 nmol/l) for insufficiency and 20 ng/ml (50 nmol/l) for deficiency[47]. A committee of the *Institute of Medicine* (IOM) of the *National Academy of Sciences* (NAS) of the USA, however, stated that 20 ng/ml (50 nmol/l) is the serum 25(OH)D level that covers the needs of 97.5% of the population and proposed this threshold for insufficiency and 12 ng/ml (30

Table 3. Determinants of clinical state (stable/recurrence)

Parameter	Coefficient	Standard error	p
Intercept	5.51	5.98	0.35
Age (years)	**-0.28**	**0.16**	**0.07 (+)**
Serum 25(OH)D concentration (ng/ml)	**-0.10**	**0.05**	**0.04 (*)**
White blood cells count (x103/µl)	**-1.14**	**0.62**	**0.06 (+)**
Neutrophil count (x103/µl)	**1.68**	**0.98**	**0.08 (+)**
Eosinophil count (x103/µl)	1.86	2.91	0.52
Eosinophil / neutrophil ratio	-6.73	8.23	0.41
Neutrophil / lymphocyte ratio	-0.39	0.41	0.35
Hemoglobin concentration (g/dl)	0.21	0.46	0.63

Subject parameters were introduced in the first step of logistic regression.
Clinical state: stable=0; recurrence=1.

Regression model analysis	Result
Cox-Snell R^2	R^2=0.37
Nagelkerke R^2	R^2=0.49
Hosmer-Lemeshow Goodness of Fit test	p=0.74

nmol/l) for deficiency[44]. In the current work we used the threshold of 20 ng/ml to differentiate "sufficient" vs. "non-sufficient" 25(OH)D serum levels in children since lower concentrations are considered non-safe by both classifications.

In the group of children with recurrent wheezing or asthma the measured serum vitamin D concentrations were under 30 ng/ml in approximately three quarters and under 20 ng/ml in more than a quarter of the examined subjects. These results are in accordance with other published data that show a deficit of vitamin D in children with recurrent wheezing or asthma[35,36,41-43].

Serum 25(OH)D concentration as a categorical variable was associated with gender, clinical state, month of measurement, eosinophil count, eosinophil/lymphocyte ratio and serum phosphate level (Table 1).

The detected association between serum vitamin D and phosphate level is expected, being explained by the metabolic effects of vitamin D[18]. The connection with the month of measurement is also not surprising; in temperate region countries like Romania vitamin D serum levels show strong seasonal variation in relation with sun radiation[48].

The association of vitamin D levels with blood eosinophil count found in the studied subjects supports a role of vitamin D in determining the clinical severity of asthma. In our study the subjects with low 25(OH) D serum concentrations had significantly lower eosinophil count and eosinophil/lymphocyte ratio. Data in the literature are not consonant regarding the relationship between serum vitamin D and blood eosinophil count. Some investigators found no association[49], while others found an inverse association between the two parameters in childhood asthma[35]; vitamin D was shown in *in vitro* studies to upregulate factors that enhance eosinophil migration[50,51].

In the present study the recurrent wheezing or asthma state (stable vs. recurrence) showed an association with several subject parameters. The association was significant with age, neutrophil count, eosinophil/neutrophil ratio and neutrophil/lymphocyte ratio and suggestive with serum 25(OH)D concentration, white blood cells count, eosinophil count and hemoglobin concentration (Table 2). However, among these parameters, in binomial regression analysis only vitamin D was a significant determinant of clinical state, while age, white blood cells count and neutrophil count were only suggestive determinants (Table 3).

The obtained results confirm the hypothesis of a significant role of vitamin D as a predictor of recurrence of wheezing or asthma in children (lower vitamin D levels being associated with higher recurrence rate). They also suggest that seasonality of sun exposure significantly influences vitamin D levels and subsequently the recurrence pattern.

Asthma is a group of disease entities with common clinical features such as intermittent respiratory symptoms (wheezing, tightness, cough, dyspnea), reversible airway obstruction and bronchial hyper-responsiveness[52]. Several asthma phenotypes have been described in children. A current classification of childhood wheezing based on clinical and immune criteria identified three main phenotypes: transient wheezing in infancy, non-atopic persistent wheezing and immunoglobulin E-associated/ atopic persistent wheezing[53,54]. The described kinetics of the three phenotypes in developed countries suggest a different age distribution: transient wheezing is limited to infancy, non-atopic persistent wheezing to preschool years, while atopic persistent wheezing extends up to early adolescence. The predominance of atopic asthma at the end of the first decade of life, is, however, a feature of developed regions; a hi-

gher prevalence of non-atopic wheezing at this age has been found in underdeveloped areas[55].

Lower respiratory infection of viral etiology is the most prevalent cause of wheezing in children. It is associated with neutrophilia[56,57] and with the development the non-atopic persistent type of wheezing[58]. The results of the present study, which show a direct association between wheezing or asthma recurrence and neutrophilia in children, are in accordance with the described predominance of respiratory infection-related recurrence of wheezing in this age group.

Medication alters the immune parameters in asthma. Inhaled corticosteroid use exerts opposing effects on airway immune effectors: it enhances both eosinophil apoptosis[59] and neutrophil survival[60]. We did not test for the airway inflammation in our study. We haven't detected a significant link between blood eosinophil and neutrophil changes and inhaled corticosteroid use, which is in accordance with the findings of other investigators[61].

We did not detect a significant association between maintenance corticosteroid use and respiratory state (stable state vs. recurrence of wheezing or asthma).

Limitations of the current study derive from its design: the small number of study subjects and non-uniform distribution of hospital admissions during the examined period are inherent to the choice of the study location; although the seasonality of serum vitamin D concentration is evident from the examined data, oral supplementation with vitamin D preparations was not accounted for in the study.

CONCLUSION

Recurrence of wheezing or asthma was associated with low serum 25(OH) vitamin D concentration in the studied subjects. The obtained data suggest that vitamin D deficiency plays an important role in determining wheezing and asthma recurrence in Romanian children.

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