

25-Hydroxyvitamin D, biomarkers of eosinophilic inflammation, and airway remodeling in children with newly diagnosed untreated asthma

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ABSTRACT

Background: Low 25-hydroxyvitamin D (25[OH]D) and asthma development may be related to airway remodeling and eosinophilia. Periostin is proposed as a key molecule that links remodeling and eosinophilic inflammation.

Objective: We evaluated the association of 25(OH)D concentration with periostin, peripheral blood eosinophil counts, and immunoglobulin E (IgE) in children with newly diagnosed asthma.

Methods: The study included 150 children: 110 with atopic asthma and 40 constituted a reference group. Fasting blood was collected for cell counts and serum for measurements of 25(OH)D, periostin, IgE, and C-reactive protein (CRP) concentrations.

Results: Significantly lower 25(OH)D, elevated IgE concentrations, and eosinophil counts were found in children with asthma compared with the reference group ($p = 0.0001$). A lower forced expiratory volume in the first second of expiration percentage predicted value was associated with a lower 25(OH)D value in children with asthma. The bronchodilator reversibility was inversely related to serum 25(OH)D concentrations ($R = -0.45$, $p = 0.029$). The children with asthma and with a 25(OH)D deficient concentration (≤ 20 ng/mL) had higher concentrations of periostin ($p = 0.035$) and CRP ($p = 0.01$) than those with a sufficient 25(OH)D concentration (≥ 30 ng/L). Additional analysis revealed statistically significant differences ($p = 0.013$) when comparing periostin concentrations between subjects with a 25(OH)D deficient concentration (≤ 20 ng/mL) and subjects who did not have a deficient concentration (> 20 ng/mL). In individuals with asthma, a 25(OH)D concentration of < 30 ng/mL had no impact on eosinophilia, whereas IgE concentrations were associated with increased eosinophils, and the effect of periostin on eosinophilia was small although significant. Multivariate regression, including 25(OH)D concentration, CRP level, eosinophil counts, and sex, accounted for 7% of periostin variation in subjects with asthma.

Conclusion: In newly diagnosed pediatric asthma, 25(OH)D concentrations revealed a small although significant association with periostin levels but no effect on eosinophilia. A low vitamin D concentration may increase airway remodeling induced by inflammatory mediators, but further clinical studies aimed to explain the causal link between vitamin D insufficiency and asthma are needed.

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Asthma is a chronic inflammatory disorder of the airways related to their hyperresponsiveness and remodeling.¹ Dietary habits, a high prevalence of vitamin D insufficiency, decreased physical activity

that leads to excessive body weight affect the development and expression of asthma in children.^{1–4} The current classification of asthma distinguishes several clinical phenotypes.^{5,6} Molecular phenotypes vary according to the overexpression of specific factors: periostin, serpin peptidase inhibitor B2, transforming growth factor $\beta 2$, and vascular endothelial growth factor, whereas cellular phenotypes differ in the distribution of eosinophils and neutrophils in the sputum.^{5,7} Patients with asthma and with a high T-helper (Th) 2 response may have higher airway hyperresponsiveness, eosinophilia, a higher serum IgE level, and biomarkers of airway remodeling, and benefit from different types of treatment. Those with a low Th2 response and neutrophilia are more resistant to treatment.^{6,8,9} Recently, peripheral blood eosinophil counts, fractional exhaled nitric oxide, and serum periostin

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concentration were described as surrogate biomarkers associated with distinct asthma phenotypes.⁵ Other possible novel biomarkers of inflammation and remodeling that were recently related to asthma are chitotriosidase and chitinase-like protein YKL-40.^{10,11} Periostin is an extracellular matrix protein inducible in airway epithelial cells by interleukin 4 and interleukin 13, and secreted by lung fibroblasts induced by transforming growth factor β .⁵ Periostin plays a role in the process of growth, differentiation, and remodeling, and may be engaged in the pathogenesis of fibrosis and eosinophilic airway inflammation.^{5,6,12}

Results of epidemiologic studies indicated that 25-hydroxyvitamin D (25[OH]D) deficiency may be related to the development of asthma and the frequency of asthma symptoms.^{13–15} Due to immunomodulatory properties, vitamin D may be associated with respiratory infections and possibly with early airway remodeling. Vitamin D deficiency may weaken pulmonary defenses against respiratory infections. In an observational study, 25(OH)D insufficiency was associated with an increased risk of asthma exacerbations in children.¹⁶ The role of vitamin D as a predictor of atopy and asthma-associated phenotypes has also been investigated.¹⁷ Evaluation of the association between vitamin D deficiency and asthma must take into consideration the relationship with increased airway responsiveness, eosinophil counts and serum IgE levels, and the role of 25(OH)D in airway remodeling.

This process occurs early in asthma, as a consequence of the production of mediators and growth factors by inflammatory and resident bronchial cells. Typical features of structural changes in airways are subepithelial fibrosis and airway smooth-muscle cell hypertrophy. The effects of vitamin D on controlling subepithelial fibrosis were demonstrated in the animal models and cultured human bronchial epithelial cells.^{18,19} Recent research in human fetal airway smooth-muscle cell models demonstrated that 1,25-dihydroxyvitamin D₃ not only attenuated remodeling but inhibited proliferation induced by inflammatory mediators in the developing airways.²⁰ The association of airway remodeling with low vitamin D levels was observed in severe and therapy-resistant pediatric asthma^{21,22} but has not been studied in the newly diagnosed childhood asthma. When taking into account the global 25(OH)D insufficiency in a pediatric population,^{17,23} we aimed to evaluate the association of serum 25(OH)D with markers that reflect eosinophilic airway inflammation, such as blood eosinophils, serum IgE, and periostin, in children with newly diagnosed untreated asthma.

Study Participants

The study included children ages 2–12 years who were referred to the Department of Pediatrics, Pneu-

monology and Allergology of the Children's Hospital (Bydgoszcz) and to the Department of Pediatric Pulmonology and Allergology (Warsaw) because of asthma-like symptoms. The subjects were recruited between October 2012 and March 2014. Informed parental consent was obtained, and the study protocol was approved by the bioethics committee at the Collegium Medicum (Bydgoszcz). G. Sypniewska and M. Krıntus contributed equally to the present study.

The diagnosis or exclusion of asthma was performed by experienced pediatric pulmonologists (B.S.-J., R.S.-K., A.Z.-K.) based on the patient's clinical symptoms and medical history according to the Global Initiative for Asthma^{1,24}; in children < 5 years of age, the diagnosis was based on the clinical symptoms and family history, exclusion of other than asthma causes of the bronchi obstruction; moreover, skin tests and/or the total IgE/specific IgE measurement and Asthma Predictive Index were performed. In children > 5 years of age, the diagnosis was based on the medical history, clinical symptoms, and spirometry measurement. Atopy was defined as a positive skin-prick test result and/or positive specific serum IgE to at least one food allergen or airborne allergen. All the children under study did not receive any vitamin D supplementation beyond their regular dietary intake.

Atopic asthma was diagnosed in 110 children. Our reference group included 40 children referred to the outpatient clinic for routine checkups; in these children, asthma was excluded by experienced pediatricians (B.S.-J., R.S.-K., A.Z.-K.) based on a thorough patient history, which indicated the low likelihood of asthma, and skin tests and/or total IgE/specific IgE measurements were performed to exclude allergy. Children with any underlying liver, kidney, or endocrine diseases, or who were receiving drugs that affected vitamin D levels were excluded. Overweight or obesity was established based on body mass index (BMI) for age percentiles.²⁵ Children with a BMI between the 85th and 95th percentile were considered overweight, whereas those with a BMI of >95th percentile were considered obese.

Spirometry and Bronchodilator Reversibility Measurements

Spirometry was performed by using Lungtest 1000 spirometer (MES, Cracow, Poland) in children > 6 years of age (30% of children with asthma who were involved in this study). The following parameters were analyzed: the forced expiratory volume in the first second of expiration percentage predicted value (FEV₁%) and the Tiffeneau index. When the Tiffeneau index was below the lower limit of normal (*i.e.*, –1.645 SD), bronchodilator reversibility (BDR) was measured after administration of 2.5 mg or 400 μ g salbutamol to

Table 1 Biochemical and clinical characteristics of study participants

	Atopic Asthma (<i>n</i> = 110)	Reference Group (<i>n</i> = 40)	<i>p</i> Value
Sex, M/F, %	53.4/46.6	57.1/42.9	0.600
Age, median (IQR), y	5.0 (4.0–7.5)	4.5 (3.8–7.0)	0.130
BMI, median (IQR), kg/m ²	16 (15.0–17.0)	16.0 (14.7–17.1)	0.980
25(OH)D value, mean (SD), ng/mL	27.7 ± 8.3	35.4 ± 13.04	0.0001
25(OH)D value of ≤20 ng/mL, %	18.2	10.0	0.127
25(OH)D value of <30 ng/mL, %	62.7	35.0	0.003
Periostin level, median (IQR), ng/mL	124 (120–132)	122 (113–126)	0.060
CRP value, median (IQR), mg/L	0.30 (0.09–0.70)	0.10 (0.08–0.60)	0.220
Eosinophil count, median (IQR), cells/μL	290 (180–430)	130 (90–230)	0.0001
Eosinophil count of >300 cells/μL, %	47.7	21.7	0.034
Neutrophil count, median (IQR), 10 ³ /μL	3.78 (2.64–4.51)	3.19 (2.32–4.40)	0.249
Log total IgE value, mean (SD), kU/L	2.07 ± 0.73	1.03 ± 0.5	0.0001

IQR = Interquartile range; BMI = body mass index; 25(OH)D = 25-hydroxyvitamin D; SD = standard deviation; CRP = C-reactive protein; IgE = immunoglobulin E.

determine the reversibility of airflow obstruction. BDR was defined as an increase of ≥12% in FEV₁% predicted.

Laboratory Measurements

Fasting blood samples were collected in the morning. Blood cell counts (Sysmex XE-2100; Kobe, Japan) were performed. Serum was stored deep-frozen until assayed for the total 25(OH)D concentration (IDS Ltd, Boldon, U.K.), periostin (Periostin/OSF-2; Phoenix Pharmaceuticals, Inc., Burlingame, CA), total IgE and specific IgE (ImmunoCAP; Phadia AB, Uppsala, Sweden), high-sensitivity C-reactive protein (CRP), (PENTRA 400; Horiba ABX, Montpellier, France). A 25-(OH)D assay does not show the cross-reactivity with 3-epi-25(OH)D. The detection limit was 2.5 ng/mL. Human periostin enzyme-linked immunosorbent assay has a limit of detection of 0.22 ng/mL and upper range of 54 ng/mL. A 25(OH)D concentration of ≥30 ng/mL was accepted as sufficient, 21–29 ng/mL as insufficient, and ≤20 ng/mL as deficient according to the recommendations.^{14,23,26,27} The cutoffs for the total IgE value (kU/L) were accepted in reference to age. Serum was assayed for specific IgE to the following allergens: grass mix, common silver birch, mugwort, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat dander, dog dander, horse dander, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria alternata*, egg white, egg yolk, milk, fish (cod), α-lactalbumin, β-lactoglobulin, casein, BSA (cow), wheat, rice, soybean, peanut, hazelnut, carrot, potato, and apple. A specific IgE level of ≥3.5 kU/L was considered positive. The cutoffs for blood eosinophils were the following: <300 cells/μL to define normal values, 300–500 cells/μL for intermediate, and >500 cells/μL for high values (eosinophilia). The

normal range for the neutrophil count was 1.5–8.0 × 10³/μL.

Statistical Analysis

We used descriptive statistics to obtain the characteristics of the study and reference groups. Continuous variables were expressed as mean (standard deviation) (Gaussian distribution) and medians with 25th and 75th percentiles (non-Gaussian distribution). The data were compared by using the unpaired Student's *t*-test, and analysis of variance, followed by the Tukey test for normally distributed variables and the Mann-Whitney *U* test and the Kruskal-Wallis test for non-normally distributed, with the *post hoc* Dunn tests. Natural-log transformed total IgE values were used for comparisons. The Spearman correlation, multivariate logistic regression, and linear regression analysis were performed. Age and BMI were adjusted in the models. A *p* value of <0.05 was considered significant. The statistical analysis was performed by using Statistica 10.0 for Windows (StatSoft; Tulsa, OK) and MedCalc 12.7.0 (MedCalc; Ostend, Belgium).

RESULTS

Baseline characteristics of the study participants are presented in Table 1. Children with asthma had significantly lower 25(OH)D concentrations compared with the reference group. Both groups were characterized by similar age and BMI, although the proportion of subjects who were overweight and obese was significantly higher among those with asthma. The frequency of positive specific IgE results in children with asthma was 48% compared with 5% in the reference group (*p* = 0.001). Periostin and CRP concentrations were

Table 2 Comparison of selected biochemical and anthropometric indices in relation to 25(OH)D concentration in children with atopic asthma

	Group A, 25(OH)D Deficiency (≤ 20 ng/mL) ($n = 20$)	Group B, 25(OH)D Insufficiency (21–29 ng/mL) ($n = 41$)	Group C, 25(OH)D Sufficiency (≥ 30 ng/mL) ($n = 49$)	p Value	Post hoc*
Age, median (IQR), y	6.0 (5.0–8.5)	6.0 (4.0–7.0)	4.5 (4.0–7.0)	0.005	A vs C; $p = 0.002$
BMI, median (IQR), kg/m ²	15.6 (14.6–16.0)	16.1 (15.2–17.4)	16.0 (15.0–17.0)	0.090	A vs C; $p = 0.019$
Female sex, no. (%)#	12 (60)	19 (46)	21 (43)	0.220	NS
25(OH)D value, mean $\pm$ SD, ng/mL	17.3 $\pm$ 2.4	24.2 $\pm$ 2.6	36.8 $\pm$ 5.4	<0.0001	A vs B, A vs C, B vs C; $p = 0.0001$
CRP value, median (IQR), mg/L	0.70 (0.30–3.15)	0.20 (0.09–0.60)	0.30 (0.09–0.80)	0.030	A vs B, $p = 0.002$; A vs C, $p =$ 0.01; B vs C, $p = 0.01$
Eosinophil count, median (IQR), cells/ μ L	300 (175–465)	320 (207–392)	265 (120–455)	0.440	NS
Neutrophil count, median (IQR), $\times 10^3/\mu$ L	4.67 (2.82–5.41)	4.12 (2.54–4.53)	3.45 (2.80–3.92)	0.050	A vs C; $p = 0.060$
Periostin, median (IQR), ng/mL	129 (120–150)	124 (120–130)	123 (121–131)	0.050	A vs C; $p = 0.035$
Log total IgE value, mean $\pm$ SD, kU/L	2.30 $\pm$ 0.62	1.99 $\pm$ 0.7	2.11 $\pm$ 0.81	0.099	NS

25(OH)D = 25-Hydroxyvitamin D; IQR = interquartile range; BMI = body mass index; NS = not significant; SD = standard deviation; CRP = C-reactive protein; IgE = immunoglobulin E.

*The presence of a statistically significant difference in the post hoc analysis.

#The p value for trend was calculated by using the Cochran-Armitage test.

higher, although not significantly in children with asthma. The elevated total IgE ($\log_{10}$ IgE) concentration and eosinophil counts were other characteristic features in the children with asthma.

The concentration of 25(OH)D in children with asthma was within the range of insufficiency (27.7 ± 8.3 ng/mL). Among the children with asthma, 18.2% had a vitamin D deficient concentration (≤ 20 ng/mL). The proportion of 25(OH)D concentration that was insufficient or deficient was considerably higher in the children with asthma ($p = 0.003$). Additional analysis revealed statistically significant differences ($p = 0.013$) when comparing periostin concentrations between children with a 25(OH)D concentration deficient (≤ 20 ng/mL) and children without a 25(OH)D concentration deficient (> 20 ng/mL). The 25(OH)D concentration is dependent on the season; however, we collected samples from October until April and assumed that, in this period, the vitamin D levels would be lower.

In the children with asthma, a weak but significant relationship of periostin with eosinophil counts, the

CRP concentration, and the 25(OH)D concentration was found: $R = 0.18$, $p = 0.02$; $R = 0.20$, $p = 0.009$; and $R = -0.15$, $p = 0.009$, respectively. There was an inverse correlation between 25(OH)D levels and the sum of the specific IgE values to aeroallergens ($R = -0.30$, $p = 0.05$). In children with asthma, the median (25th and 75th percentiles) predicted FEV₁% was 83 (68–88) and for BDR was 14 (13–15). BDR was inversely related to serum 25(OH)D concentrations ($R = -0.45$, $p = 0.029$). We also observed that the lower FEV₁% predicted value was significantly associated with a lower 25(OH)D level ($R = 0.27$, $p = 0.048$). A comparison of biochemical and clinical indices in relation to the 25(OH)D concentration in the children with asthma is presented in Table 2. We found significantly higher periostin and CRP concentrations in children with 25(OH)D deficiency compared with those with sufficient levels of 25(OH)D ($p = 0.035$ and $p = 0.01$, respectively). Moreover, the CRP value was significantly higher in those with a vitamin D deficiency compared with

Table 3 Predictors of eosinophilia, >300 cells/ μ L, in children with newly diagnosed atopic asthma

Parameter	Model 1*		Model 2#		Model 3§	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
Age	—		1.40 (1.15–1.71)	0.0007	1.40 (1.15–1.71)	0.001
Boys	—		—		1.29 (0.59–2.82)	0.030
BMI, kg/m	—		0.93 (0.78–1.11)	0.442	0.94 (0.79–1.12)	0.480
25(OH)D of <30 ng/mL, ng/mL	0.97 (0.93–1.01)	0.181	0.99 (0.95–1.04)	0.937	1.00 (0.96–1.04)	0.985
CRP value, mg/L	0.91 (0.79–1.06)	0.253	0.88 (0.75–1.02)	0.108	0.87 (0.75–1.02)	0.096
Periostin value, ng/mL	1.02 (1.01–1.03)	0.026	1.02 (1.01–1.03)	0.020	1.02 (1.01–1.03)	0.016
Log IgE value, kU/L	2.94 (1.74–4.97)	0.00005	2.54 (1.46–4.43)	0.0009	2.54 (1.46–4.42)	0.001

OR = Odds ratio; CI = confidence interval; BMI = body mass index; 25(OH)D = 25-hydroxyvitamin D; CRP = C-reactive protein; IgE = immunoglobulin E.

*Unadjusted; $\chi^2 = 24.86$, d.f. = 4, $p = 0.008$, Nagelkerke $R^2 = 0.20$.

#Adjusted for age and BMI; $\chi^2 = 39.36$, d.f. = 6, $p = 0.0002$, Nagelkerke $R^2 = 0.29$.

§Adjusted for age, sex, and BMI; $\chi^2 = 39.77$, d.f. = 7, $p = 0.006$, Nagelkerke $R^2 = 0.30$.

Table 4 The impact of the 25 (OH)D concentration and other variables on the periostin concentration in children with newly diagnosed atopic asthma: Regression model

Independent Variable	Adjusted R^2	F	$\beta \pm SE$	<i>p</i>
Periostin, ng/mL	0.07	2.60		0.038
25 (OH)D concentration, ng/mL			−0.494 $\pm$ 0.244	0.045
Sex			−10.51 $\pm$ 4.168	0.013
CRP level, mg/L			0.102 $\pm$ 0.914	0.911
Eosinophil count, cells/ μ L			6.097 $\pm$ 8.431	0.471

25(OH)D = 25-Hydroxyvitamin D; CRP = C-reactive protein; SE = standard error.

those with an insufficient 25(OH)D concentration ($p = 0.002$).

The impact of selected variables on eosinophilia (>300 cells/ μ L) in the children with asthma was assessed by logistic regression analysis (Table 3). Model 1, unadjusted, was highly significant and accounted for 20% of variation in eosinophilia. The IgE concentration was associated with a significant increase of eosinophilia, whereas the effect of periostin concentration was small but significant. Models 2 and 3, adjusted for BMI, age, and sex, were highly significant and accounted for 29 and 30%, respectively of variation in eosinophilia. In these models, the IgE level was associated with significant eosinophilia, whereas the impact of periostin was small, although significant. A vitamin D value of <30 ng/mL had no direct effect on eosinophilia. A multivariate regression model for the prediction of periostin variation, relied on the assumption that the dependent variable correlates with the explanatory variables (Table 4). The model included the 25(OH)D concentration, CRP value, eosinophil count, and sex, and was significant, and accounted for 7% of periostin variation; 25(OH)D concentration and sex contributed significantly; and sex seemed to be an even stronger predictor of periostin levels.

DISCUSSION

The vitamin D status and its association with asthma in children have been studied earlier, but the underlying mechanisms that show the impact of low vitamin D level on the development of asthma still remain to be elucidated.²⁸ A study performed on human fetal cell models demonstrated that the active vitamin D metabolite (calcitriol) alters airway remodeling induced by proinflammatory cytokines and could possibly alleviate changes in pediatric inflammatory airway diseases.²⁰ Periostin has been implicated in the pathogenesis of allergic diseases, regarded as a key molecule that links airway remodeling and Th2 eosinophilic inflammation.²⁹

We evaluated the association of serum 25(OH)D with the following markers of eosinophilic inflammation: the peripheral blood eosinophil count, serum IgE level, and periostin level. We demonstrated that, in children with newly diagnosed atopic asthma, the impact of the 25(OH)D concentration on periostin level variation was small but significant. On the contrary, insufficient or deficient vitamin D levels had no significant effect on eosinophilia, whereas the IgE concentration was associated with marked (>2.5-fold) and periostin with

a slight but significant increase of eosinophils. Periostin contributes to subepithelial thickening in airways of those with asthma, and its expression in airway epithelium was correlated with airway basement membrane thickness.³⁰

Periostin may be a potentially useful biomarker of airflow limitation, as a consequence of airway remodeling, in adult patients with asthma who were treated with inhaled corticosteroids.³¹ In children with asthma and with mild disease, the subepithelial airway remodeling was assessed by using *in vitro* cultured airway epithelial cells.⁷ The expression of transforming growth factor β 2 and periostin in cultured bronchial epithelial cells from children with asthma was significantly higher than in healthy subjects, which indicated that periostin might play a role in the development of subepithelial airway remodeling in children with asthma. Periostin production in the airways is well reflected by its serum level, and, thus, periostin can be regarded as a potentially useful biomarker of airway remodeling and Th2 eosinophilic inflammation in the individuals with asthma.^{29,32,33}

Vitamin D deficiency early in life may contribute to increased eosinophilic inflammation and airway remodeling in childhood asthma.²⁴ A higher serum periostin level in children ages 6–15 years and with asthma was found to be associated with airway hyperresponsiveness to methacholine and mannitol.³⁴ In our study, however, vitamin D deficiency was found only in 18.2% of children with asthma compared with 10% in the reference group, which may explain the lack of significant difference in periostin concentration. We observed that BDR was inversely related to serum 25(OH)D concentrations and that the lower FEV₁% predicted value was significantly associated with a lower 25(OH)D level, which may indicate that lower 25(OH)D levels are related to airway function and remodeling.²² Noteworthy, the children with a 25(OH)D concentration deficient and newly diagnosed atopic asthma showed a significantly higher serum periostin level and CRP concentration than the children with a sufficient 25(OH)D concentration.

A higher CRP level in children with a 25(OH)D deficient concentration in our study may indicate an effect of inflammation on circulating vitamin D concentrations. The 25(OH)D concentration may be considered a negative acute-phase reactant and its lower levels are the result not the cause of systemic inflammation, as proposed by Brehm,³⁵ which may be an alternative explanation for the lack of a direct impact of a low 25(OH)D value on eosinophilia observed by us. We had no possibility to evaluate more-recognized markers of eosinophilic inflammation, *e.g.*, fractional exhaled nitric oxide, and other beyond periostin, markers of airway remodeling. Nevertheless, we are aware of the report on serum periostin that shows that it is

less variable than fractional exhaled nitric oxide in patients with asthma.²⁹ Moreover, in this study, we did not assess serum chitinases, which have been proposed as possible novel biomarkers of airway inflammation and remodeling, particularly in severe pediatric therapy-resistant asthma.¹⁰

Multivariate regression analysis demonstrated that 25(OH)D concentration and sex were associated with serum periostin. Both 25(OH)D concentration and sex significantly predicted the periostin level variation in patients with asthma. Sex-specific differences in the pathogenesis of severe asthma, especially airway remodeling, have been studied, mostly in mouse models, and the results were inconsistent.^{36,37} In humans, sex-specific differences in periostin concentrations or expression were assessed only in older patients with heart failure or after aortic valve replacement.^{38,39} Sex-specific differences were observed in the concentrations of biomarkers of remodeling, and serum periostin value was found to be significantly higher in men.³⁸

The serum periostin level has been indicated as a useful biomarker of eosinophilic inflammation and airway remodeling in adults with asthma, but its usefulness in the pediatric population still needs explanation and verification. There presently are very little data on the levels of this protein in relation to age in a healthy population. The recent report by Inoue *et al.*⁴⁰ showed a higher periostin concentration in healthy children ages 6–11 years than in adults and that the presence of allergic conditions in children does not increase its level. This may explain the lack of significant differences among the groups in our study. Our study showed that concentration of periostin was only slightly higher in boys with atopic asthma compared with girls. It, however, is difficult to draw conclusions, and we may only suggest that sex-related differences in remodeling in asthma may be influenced by the different patterns of secretion of sex hormones.

Vitamin D insufficiency may have some implications in pertaining to changes in lung function because early childhood is when the respiratory system is particularly sensitive to environmental and nutritional influences.⁴¹ Analysis of data from epidemiologic studies indicated that a low 25(OH)D concentration may contribute to symptoms and morbidity rates in patients with asthma.^{17,42,43} A recent meta-analysis that summarized the evidence from epidemiologic studies of 25(OH)D concentration deficiency or insufficiency and the risk of pediatric asthma in different ethnic populations indicated that it might increase the risk.⁴⁴ Contrary to this, another study, on Asian children and adolescents, provided epidemiologic evidence against the association of 25(OH)D status with concurrent asthma, atopy, and total IgE values.⁴⁵ Recent systematic reviews and a meta-analysis on vitamin D supplementation in children with asthma indicated that there

is not enough evidence yet for confirming or ruling out beneficial effects of supplementation in children with asthma.^{46–48} Whether vitamin D represents a novel preventive and/or therapeutic strategy still needs clarification. Well-designed clinical trials on vitamin D supplementation are necessary. As there is no consensus, it seems reasonable to consider vitamin D supplementation in patients with asthma and with low 25(OH)D concentrations.

Several limitations of our study should be acknowledged. Lung function tests (predicted FEV₁% and BDR) were performed only in children > 6 years of age and not in the reference group. Although we recognize the importance of spirometry, there currently is no criterion standard test available for asthma diagnosis, which is principally based on a thorough patient history undertaken by an experienced clinician.⁴⁹ We measured traditional biomarkers related to eosinophilic inflammation and periostin, a candidate biomarker of airway remodeling. As did other researchers,⁶ we used peripheral blood eosinophils to quantify eosinophilic inflammation because this method is simple, noninvasive, and not expensive, even though this method does not correlate well with elevated sputum eosinophil levels in patients with asthma.^{50,51} Moreover, we did not measure airway muscle mass or basement membrane thickening to objectively assess airway remodeling. A further limitation that should be acknowledged is that, although children with asthma had significantly lower 25(OH)D concentrations compared with the reference group, neither group was deficient (<20 ng/mL). In addition, the differences in periostin levels reported in this study were small although significant, accordingly, the clinical significance of these differences remains undetermined.

CONCLUSION

We concluded that, in newly diagnosed pediatric asthma, 25(OH)D concentrations revealed a small although significant association with periostin levels but had no effect on eosinophilia. Low vitamin D concentrations may increase airway remodeling induced by inflammatory mediators, but further clinical studies are needed to explain the potential causal link between vitamin D insufficiency and asthma.

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