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Decreased Serum Vitamin D Levels in Children with Asthma are Associated with Increased Corticosteroid Usage

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Abstract

Background—There is little knowledge about clinical variables associated with vitamin D (vitD) insufficiency in asthmatic children.

Objective—To investigate disease variables associated with vitD insufficiency in childhood asthma and interaction of vitD with corticosteroid-mediated anti-inflammatory responses.

Methods—We analyzed 25-hydroxyvitamin D serum levels in 100 asthmatic children to investigate relationships between 25-hydroxyvitamin D levels and patient characteristics. We determined vitD effects on dexamethasone (DEX) induction of mitogen-activated protein kinase phosphatase-1 (MKP-1) and IL-10 in peripheral blood mononuclear cells (PBMC).

Results—The median 25-hydroxyvitamin D serum level was 31 ng/mL. 47% of subjects had vitD levels in the insufficient range (<30 ng/mL), while 17% were vitD deficient (<20 ng/mL). Log₁₀ IgE ($p=0.01$, $\rho=-0.25$) and the number of positive aeroallergen skin prick tests ($p=0.02$, $\rho=-0.23$) showed a significant inverse correlation with vitD, whereas FEV₁% predicted ($p=0.004$, $\rho=0.34$) and FEV₁/FVC ratio ($p=0.01$, $\rho=0.30$) showed a significant positive correlation with vitD. The use of inhaled steroids ($p=0.0475$), oral steroids ($p=0.02$), and total steroid dose ($p=0.001$), all showed significant inverse correlations with vitD. The amount of MKP-1 and IL-10 mRNA induced by vitD plus DEX was significantly greater than that induced by DEX alone ($p<0.01$). In an experimental model of steroid resistance where DEX alone did not inhibit T cell proliferation, addition of vitD to DEX resulted in significant dose dependent suppression of cell proliferation.

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Conclusions—Corticosteroid use and worsening airflow limitation is associated with lower vitD serum levels in asthmatics. VitD enhances glucocorticoid action in asthmatic PBMC and enhances the immunosuppressive function of DEX *in vitro*.

Clinical Implications—Our study suggests that vitD supplementation may potentiate anti-inflammatory function of corticosteroids in asthmatics and thereby improve asthma control.

Keywords

vitamin D; children; asthma

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways which causes an increase in airways hyperresponsiveness leading to recurrent episodes of wheezing, breathlessness, and coughing that are associated with variable airflow obstruction.¹ According to data from the National Health Interview Survey, as of 2007, 8 million children between the ages of 5 and 17 have been diagnosed with asthma in their lifetime.² In the United States, asthma is the most common cause of childhood emergency department visits, hospitalizations, and missed school days.³ Inhaled corticosteroids (ICS) represent the preferred treatment for persistent asthma.⁴ For patients with severe asthma who do not achieve adequate control with high dose ICS therapy combined with a long-acting beta agonist (LABA), oral corticosteroids become a preferred treatment modality.⁴ While clearly effective in the management of asthma, corticosteroids come with a variety of potential side effects. Higher ICS doses increase the potential for systemic adverse effects that are seen with oral corticosteroids, including reduced bone density, increased fracture risk, and adrenal suppression.^{5, 6} Consequently, treatment options for asthma that reduce steroid dose can minimize the risk of these adverse effects.

One hypothesis for the rising prevalence of asthma involves vitD. Some have argued that different factors associated with westernization have led to lower vitD levels, which in turn has resulted in higher rates of asthma.^{7, 8} However, others have argued that vitD has more of a deleterious effect on allergic pathogenesis.⁹ Although multiple studies have examined maternal vitD status and subsequent wheezing in offspring,^{8–11} there is limited data on vitD levels in children with asthma, as well as on what features of asthma are associated with vitD levels. A recent study on children with asthma from Costa Rica showed a significant inverse association between vitD levels and use of antiinflammatory medication (either ICS or leukotriene inhibitor) in the previous year, total IgE, and eosinophil count.¹² These important findings require confirmation. To our knowledge, the prevalence of vitD insufficiency/deficiency is unknown for children with asthma living in higher northern latitudes. In addition, more information is needed regarding the specific clinical and therapeutic variables associated with lower vitD levels in childhood asthma. The first aim of this paper was to investigate the prevalence of vitD insufficiency in childhood asthma from a group of patients living in northern latitudes, as well as to further define what variables, including corticosteroid use and markers of allergy, are associated with vitD insufficiency in childhood asthma. The second part of this study was to determine whether vitD interacts directly with corticosteroid pathways that lead to down-regulation of the inflammatory response. This was assessed by testing whether vitD enhances glucocorticoid induction of MKP-1 and IL-10 in PBMCs using real-time PCR and T cell proliferation assays.

METHODS

Subjects

Children with asthma referred to National Jewish Health were identified via focused searches of laboratory data utilizing codes for the 25-hydroxyvitamin D assay. Data was collected between April 1, 2008 and October 31, 2009. Patient medical information was obtained via the National Jewish Health electronic medical record and the National Jewish Research Database.¹³ Patients between the ages of 0 and 18 who had 25-hydroxyvitamin D serum levels (used interchangeably with vitD levels in this paper) drawn were included if they had a physician diagnosis of asthma. Patients were excluded if there was documentation that they were taking vitD supplements or if they had additional chronic pulmonary conditions (such as cystic fibrosis or bronchiectasis). Laboratory studies to assess vitD effects on corticosteroid action *in vitro* utilized eleven patients with mild to moderate asthma and four normal control subjects. Approval was received from the National Jewish Health Institutional Review Board for both parts of the study.

Data Collection

Serum 25-hydroxyvitamin D levels were analyzed using the vitD, 25-hydroxy chemiluminescent immunoassay performed at ARUP Laboratories (Salt Lake City, UT). This assay is capable of measuring both D₂ and D₃ derivatives of 25-hydroxyvitamin D.¹⁴ Values were reported as ng/mL. 25-hydroxyvitamin D levels are the preferred marker of the body's vitD status as this form has a longer half-life (2–3 weeks) than 1,25-dihydroxyvitamin D (4 hours).¹⁵ Skin testing was performed according to National Jewish guidelines using histamine and saline controls. Positive reactions were recorded for wheal sizes greater than or equal to 3 mm in diameter above the negative saline control. Seasonal aeroallergens tested were specific for the plants commonly found in the subject's home state. Total IgE and eosinophil count was performed by Advanced Diagnostics Laboratories (Denver, CO) at National Jewish Health. Reflex titer assays were done to quantify IgE levels above 5,000 kU/L. IgE levels underwent a log₁₀ transformation for analysis. Eosinophil count was determined by the direct current electronic resistance method of particle counting and sizing. Latitude of the patient's home address was determined based on data from the United States Census Bureau Gazetteer website and the iTouchMap.com website. Exhaled nitric oxide was measured by the NIOX system (Aerocrine, Sweden). Medication usage and dosage was recorded. The total steroid dose was expressed as the average daily dose of inhaled plus oral corticosteroids taken over the 30 days prior to vitD assessment.

Laboratory studies were performed on purified PBMC. Human PBMCs were isolated by Ficoll-Hypaque[®] density gradient centrifugation. PBMC were cultured in hormone-free medium containing 1,25-(OH)₂D₃ (10nM) for 24 hours with dexamethasone (DEX) (10 nM or 100nM) added during the last 3 hours. Total RNA was extracted (Qiagen), transcribed into cDNA, and analyzed by real-time PCR using the dual-labeled fluorogenic probe method on an ABI Prism 7300 Real Time PCR system (Applied Biosystems). MKP-1, IL-10 and beta-actin mRNA expression was determined. For proliferation studies, PBMC were cultured in RPMI 1640 medium containing 10% Fetal Calf Serum, stimulated with staphylococcal toxic shock syndrome toxin 1 (TSST-1) (Toxin Technology Inc., Sarasota, FL) (100ng/ml) for 72 hours to induce corticosteroid resistance as described by us earlier.¹⁶ 100nM DEX, with or without 0.1nM, 1nM, 10nM, 50nM, 100nM 1,25-(OH)₂D₃ were added to examine their effects on T cell proliferation.

Statistical Analysis

Population values for the variables examined are given in Table I. Univariate relationships between 25-hydroxyvitamin D levels and patient demographic and therapeutic characteristics

were determined using Spearman's rank correlation coefficient when the variables were continuous and the Wilcoxon test with chi-square approximation when they were categorical. These tests were chosen because of the nature of the retrospective convenience sample, the variability in sample size among variables, and the non-normal distribution of variables. These univariate relationships are presented in Tables II and III. Variables were considered statistically significant at p values less than 0.05 using two-sided tests. Statistical analysis was performed using JMP 8.0.1 software (SAS Institute Inc., Cary, NC).

For the laboratory assessment of vitD effects on corticosteroid action, data were expressed as mean plus or minus the standard error of the mean (SEM). The paired t test was used to compare functional responses of pre- and post-DEX treated cells from the same donors (hence, paired). Wilcoxon matched pairs test was applied for samples that did not fit Gaussian distribution. A p value of less than 0.05 was considered statistically significant. All reported p values were based on two-sided tests.

RESULTS

Subject Characteristics

25-hydroxyvitamin D levels and clinical features were analyzed in a total of 100 children with asthma ages 0 to 18 years (Table I). Racial data was available for 81 of the subjects. Seventy-nine per cent of the participants were white, 9% were American Indian or Alaska Native (including Hispanic), 6% were African American, 3% were Asian, and 4% reported mixed race (data not shown).¹⁷ The median latitude was 39.0° N. The median eosinophil count and total IgE were 311 cells/mm³ and 1440 kU/L, respectively. The median for log₁₀ IgE was 7.3. The median FEV₁% predicted was 93.8% and the median FEV₁/FVC ratio was 0.8. The date 25-hydroxyvitamin D level was obtained was recorded in order to assess seasonal variations in the frequency of specimen collection. Subjects were broken down into a summer (March through October) or winter (November through February) designation. November through February was chosen for the winter season since very little vitD can be produced via sun exposure during these months in latitudes above 35° N.¹⁸ Seventy-nine per cent of the collections were done during months when cutaneous vitD synthesis was possible in northern latitudes (March through October).

Among all the asthmatics studied, the median serum 25-hydroxyvitamin D level was 31 ng/mL (see Figure 1). Based on changes in parathyroid hormone levels and intestinal calcium transport that have been noted at vitD levels less than 30 ng/mL,¹⁸ values less than 30 ng/mL were considered vitD insufficient. Forty-seven percent of the subjects studied had vitD levels in the insufficient range (less than 30 ng/mL), while 17% were vitD deficient (less than 20 ng/mL). Among the vitD deficient patients, 88% had a 25-hydroxyvitamin D level between 10 ng/mL and 20 ng/mL.

Clinical Associations

Tables II, III, and IV outline the results of the univariate analysis for variables associated with vitD levels. Tables II and III involve clinical variables. Table IV involves therapeutic (i.e. medication) variables. A significant inverse correlation was noted for age and vitD level ($p < 0.0001$, $\rho = -0.47$). The median vitD value for females was 33 ng/mL and 30 ng/mL for males. African Americans had a median vitD value of 24 ng/mL, while subjects that were not African American had a median value of 32 ng/mL. The differences in vitD with respect to gender and race were not significant, although only 6 subjects reported African ancestry. Latitude and time of year (summer versus winter) were not significantly associated with vitD level (data not shown). Higher body mass index (BMI) was associated with significantly lower vitD levels ($p = 0.04$, $\rho = -0.21$). Among markers for atopy, log₁₀ IgE ($p = .01$, $\rho = -0.25$) and

the number of positive environmental skin prick tests ($p = 0.02$, $\rho = -0.23$) showed a significant inverse correlation with vitD, while eosinophil count demonstrated no significant correlation. Sixty-nine of the participants had recorded spirometry, with FEV₁% predicted ($p = 0.004$, $\rho = 0.34$) and FEV₁/FVC ratio ($p = 0.01$, $\rho = 0.30$) showing a significant positive correlation with vitD levels.

Separate univariate analyses were performed on individual aeroallergens and aeroallergen categories (see Table III). Sensitivity to outdoor pollens was the most common skin test finding (82% of subjects) and was not associated with vitD levels. Mold allergens and *Alternaria*, in particular, showed a trend towards lower vitD levels that did not achieve significance. However, sensitivity to the indoor aeroallergens dog ($p = 0.045$) and house dust mites ($p = 0.05$) were significantly associated with lower vitD levels.

Among the different therapeutic modalities assessed (Table III), the use of inhaled steroids ($p = 0.0475$), oral steroids ($p = 0.02$), total steroid dose ($p = 0.001$), and long acting beta agonists ($p = 0.0007$) all showed significant association with lower vitD levels. The use of leukotriene receptor antagonists, topical steroids, and multivitamins were not associated with vitD levels, although only six patients reported multivitamin use.

Laboratory assessment of vitD effects on corticosteroid action

In unfractionated PBMC, DEX induced MKP-1 expression (mean±SEM) from 0.91 ± 0.17 ng MKP-1/ng β -actin for media to 8.51 ± 1.71 ng MKP-1/ng β -actin for DEX cultured cells, respectively ($n=11$). There was a significant ($p < 0.001$) enhancement of DEX induction of MKP-1 by vitD (Figure 2A). Steroid sparing effects of vitD were noted, as the amount of MKP-1 mRNA induced by vitD+10nM DEX combination was significantly greater than the amount induced by 100nM of DEX alone ($p < 0.01$) (Figure 2A). Similar effects were observed for IL-10 (Figure 2B).

To examine whether vitD can improve glucocorticoid action under corticosteroid resistant conditions, PBMC were stimulated with TSST-1 (100ng/ml) for 72 hours to induce corticosteroid resistance, and DEX (100nM) or 1,25-(OH)₂D₃ (up to 100nM) were added alone or in combination to examine their effects on T cell proliferation. TSST-1 stimulated T lymphocyte proliferation in PBMC was not inhibited by DEX alone or vitD alone. However, addition of vitD to DEX resulted in significant dose-dependent suppression of TSST-induced cell proliferation and up to 64% inhibition of cell proliferation was achieved ($p < 0.01$) (Figure 3).

DISCUSSION

In our population of pediatric asthmatics, primarily from latitudes above 35° N, the prevalence of vitD insufficiency (<30 ng/mL) was 47%, with 17% of patients vitD deficient (<20 ng/mL). These percentages were higher than a recent study involving childhood asthma and vitD that was done in an equatorial population from Costa Rica, where 28% had vitD insufficiency.¹² The majority of our patients (79%) had vitD levels collected during a time period where cutaneous production of vitD is possible in higher northern latitudes (March through October). The differences between our study and the population from Costa Rica supports the known association of increased risk of vitD deficiency in populations living at higher northern latitudes.¹⁵ A study in infants and toddlers ages 8–24 months from an urban population where the majority had darker skin pigmentation found that 40% of the participants were vitD insufficient (≤ 30 ng/mL).¹⁹ In an adolescent urban population again with a majority of subjects with darker skin pigmentation, 42% of the participants had vitD levels less than 20 ng/mL.²⁰ Both of these studies were done on populations residing in northern latitudes. A recent paper describing 25-hydroxyvitamin D levels in children utilizing the National Health and Nutrition

Examination Survey from 2001–2004 showed that 61% of children ages 1–21 had insufficient levels of vitD.²¹ Overall, the prevalence of vitD insufficiency in children with asthma from our study population are similar to general pediatric populations living at similar northern latitudes.

Our univariate analysis demonstrated several statistically significant variables. Age and BMI both had significant inverse correlations with serum vitD levels. These findings are consistent with prior data.^{18, 21} In terms of lung function, FEV₁% and FEV₁/FVC ratio were also significantly correlated with vitD level. In a study using an adult NHANES sample of the general population, subjects whose vitD level was in the highest quintile had significantly higher FEV₁ and FVC.²² Some elements of atopy, such as log₁₀ IgE level and the number of positive aeroallergen skin tests, were inversely correlated with vitD levels in serum, while eosinophil count was not. The relationship found between aeroallergen sensitivity and lower vitD levels appears to be driven by perennial aeroallergens, house dust mite and dog in particular. Clinically, correlations between lower vitD levels and markers of allergy in childhood asthma have also been found by other investigators.¹² With respect to anaphylaxis, higher epinephrine autoinjector prescription rates have been shown in northern latitudes (a presumed area of low vitD levels) after controlling for socioeconomic factors.²³

Importantly, our study also demonstrated significant associations between inhaled corticosteroids, oral corticosteroids use, and total steroid dose with lower vitD levels. Our data is consistent with a previous report of lower 25-hydroxyvitamin D levels in patients taking daily oral glucocorticoids at doses of 15–100 mg.²⁴ Cumulative glucocorticoid dose exposure has been associated with lower 25-hydroxyvitamin D levels in a more recent study on patients with systemic lupus erythematosus.²⁵ One explanation for our findings is that lower vitD levels contribute to increasing asthma severity and a concomitant need for escalating pharmacologic intervention, which frequently will entail inhaled and oral glucocorticoid administration. An additional novel possibility we considered is that vitD has effects on glucocorticoid pathways and vitD insufficiency promotes the need for higher doses of glucocorticoids to achieve treatment effect.

Upregulation of MKP-1 and IL-10 expression is essential for glucocorticoid mediated anti-inflammatory and immunosuppressive effects. Xystrakis and colleagues reported that the addition of vitD and DEX to cultures of CD4⁺ T regulatory cells from steroid resistant asthmatics enhanced IL-10 secretion from these cells to levels comparable from cells of steroid sensitive patients treated only with DEX.²⁶ The study also demonstrated that vitD blocked DEX induced downregulation of the glucocorticoid receptor in CD4⁺ cells from healthy volunteers.²⁶ When three steroid resistant subjects were given small doses of vitD for seven days, all subjects showed enhanced IL-10 expression by CD4⁺ cells.²⁶ This important finding has yet to be confirmed by other investigators.

In the current study, we tested whether vitD enhances glucocorticoid induction of MKP-1 and IL-10 in PBMC using real-time PCR and T cell proliferation assays. The data suggests vitD enhancement of glucocorticoid action in asthmatic PBMC *in vitro*. Similarly, significant enhancement in DEX-induced MKP-1 and IL-10 mRNA was observed following vitD pretreatment in PBMC from normal healthy control subjects, suggesting that vitD effect was not exclusive for asthmatics.²⁷ Also, *in vitro* data demonstrates that vitD addition can lower the active dose of DEX greater than ten-fold. In an experimental model of steroid resistance induced by staphylococcal superantigen, presence of vitD in culture restored immunosuppressive function of DEX. The results of this study suggest that vitD supplementation may potentiate anti-inflammatory function of corticosteroids in asthmatics.

Our study has several limitations. First, our clinical data was retrospective and relied on electronic medical record documentation. Given the emerging data on possible relationships between vitD and allergic diseases,^{7,23,28} patients entering our day program during the study period routinely had vitD levels drawn irrespective of provider concerns for rickets or dietary deficiencies. This minimized a bias towards lower vitD levels. Second, the variability in the data, the sample size, and the non-normal distribution of several variables made it difficult to create an efficient multivariate model to examine the strength of the correlations noted in our univariate analyses. Third, our small sample size and the tertiary nature of our institution may limit the generalization of these findings to all U.S. children with asthma. One advantage of our data set is our patient's live in multiple locations. Even though our median latitude (39°N) was indicative of our institution's location, only 30% of our subjects resided in Colorado and our data set included patients from a total of 28 different states representing a variety of northern latitudes (18–49°N).

In summary, our study provides important information on vitD levels and childhood asthma in higher northern latitudes. We were able to show that vitD insufficiency in childhood asthma is common and similar to the general population. We found significant correlations between several markers of atopy and lung function with vitD levels. Our study involved a high number of oral steroid dependent asthmatics and demonstrated significant correlations between inhaled steroid usage, oral steroid usage, and total steroid dose with vitD levels. This finding supports our laboratory findings that vitD enhances the anti-inflammatory effects of glucocorticoids. These findings have important implications on potential future directions in asthma research. First, these findings should be confirmed in a prospective fashion that involves the generation of an efficient multivariate model. Second, further research should be directed at the use of vitD supplementation as a potential steroid sparing agent in moderate to severe persistent asthma, as well as a modifier of asthma disease severity.

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ABBREVIATIONS

DEX	Dexamethasone
ICS	Inhaled corticosteroids
LABA	Long-acting beta agonist
MKP-1	Mitogen-activated protein kinase phosphatase-1
PBMC	Peripheral blood mononuclear cells
SEM	Standard error of the mean
TSST	Toxic shock syndrome toxin
VitD	Vitamin D
VDR	VitD receptor

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Figure 1. Distribution of vitD Levels. Vitamin D levels are expressed as serum 25-hydroxyvitamin D. The median value was 31 ng/mL. Forty-seven per cent of asthmatics had insufficient levels of vitD (less than 30 ng/mL).

Figure 2A

Figure 2B

Figure 2.

Vitamin D (vitD) potentiated DEX-mediated transactivation in human PBMC: enhances DEX induction of MKP-1 (**A**) and IL-10 (**B**) mRNA. PBMC from patients with asthma were cultured with 10nM vitD or media alone for 24h and supplemented with 10 or 100 nM of DEX for the last 3h of culture. MKP-1 mRNA levels were detected by Real-Time PCR, and were normalized to actin mRNA. Values represent mean $\pm$ SEM (n=11).

Figure 3.

Vitamin D augments DEX effect on TSST-1 stimulated lymphocyte proliferation in human PBMC. PBMC from normal control subjects were treated with DEX and vitD as indicated in methods. ^3H -thymidine was added to the medium 18h before collecting the cells. Cell division was estimated based on ^3H -thymidine incorporation (n=4).

Table I

Patient Characteristics

Characteristic	Sample Size	Data *
Age, years	100	7 (4–10)
Gender	100	Male 64%
BMI percentile	98	60 (29.5–85.6)
Eosinophil count, cells/mm ³	91	311 (146–576)
Log ₁₀ IgE	96	7.3 (6–9)
IgE, kU/L	96	1441 (386–7678)
Number of positive aeroallergen skin tests	96	9 (4–16)
FVC%	69	99 (90.3–106.8)
FEV ₁ %	69	93.8 (80.9–101.8)
FEV ₁ /FVC	69	0.8 (0.74–0.87)
Season	100	Summer 79%

* Values listed as percent of population or median (25th–75th interquartile range)

Table IIUnivariate analysis of associations between serum vitD levels and continuous clinical variables^{*}

Variable	Correlation [*]	P value
Age	−0.47	<0.0001
BMI [†]	−0.21	0.04
Eosinophil count	−0.01	0.91
Log ₁₀ IgE	−0.25	0.01
Number of positive aeroallergen skin tests	−0.23	0.02
FVC%	0.12	0.33
FEV ₁ %	0.34	0.004
FEV ₁ /FVC	0.30	0.01

^{*} Correlation was determined with Spearman's rank correlation coefficient[†] Body Mass Index

Table IIIUnivariate analysis of serum vitD levels and aeroallergen sensitivity^{*}

Allergen	Skin Test Positive (%)	Vitamin D Level [†] Median (IQR)	P value [‡]
Pollen N = 93	82	Positive: 29 (22–40) Negative: 31 (23–36)	0.94
Dog N = 95	63	Positive: 29 (23–37) Negative: 35 (22–47)	0.045
Cat N = 95	60	Positive: 29 (23–38) Negative: 31.5 (26–41)	0.47
Mold N = 95	55	Positive: 28 (22–38) Negative: 34 (23–42)	0.09
House Dust Mite N = 92	44	Positive: 27 (16–39) Negative: 31 (27–38)	0.05
Alternaria N = 93	39	Positive: 27.5 (20–34) Negative: 33 (25–40)	0.06

^{*} Positive aeroallergen sensitivity is defined as a positive skin test to the allergen in question or any one positive skin test to a specific allergen within the group in question.

[†] Vitamin D represents 25-hydroxyvitamin D. Categorical variables have comparison vitD medians expressed in ng/mL with 25th–75th interquartile ranges in parentheses.

[‡] The Wilcoxon test was used for median value differences for categorical variables.

Table IV

Univariate analysis of serum vitD levels and medication use

Medication Used, Sample Size (n= 100)	Vitamin D Level [†] Median (IQR)	P value [*]
Inhaled corticosteroid N = 60	ICS [§] : 29 (21–36) No-ICS: 35 (26–42)	0.0475
Oral corticosteroid N = 14	Oral CS: 25 (18–30) No-Oral CS: 32 (25–40)	0.02
Topical corticosteroid N = 69	TCS: 31 (23–40) No-TCS: 31 (24–38)	0.98
LTRA N = 42	LTRA: 29 (25–37) No-LTRA: 32 (23–41)	0.50
LABA N = 29	LABA: 25 (19–31) No-LABA: 34 (27–42)	0.0007
MVI N = 6	MVI: 40 (27–53) No-MVI: 31 (23–39)	0.12
Total steroid dose (mg) [‡] N = 64	ρ : -0.32 [#]	0.001

^{*} The Wilcoxon test was used for median value differences for categorical variables. Correlation was determined with Spearman's rank correlation coefficient for continuous variables.

[†] Vitamin D represents 25-hydroxyvitamin D. Categorical variables have comparison vitD medians expressed in ng/mL with 25th–75th interquartile ranges in parentheses.

[‡] Total steroid dose includes inhaled plus oral corticosteroids, mg = milligrams

[§] ICS = inhaled corticosteroid, Oral CS = oral corticosteroid, TCS = topical corticosteroid, LTRA = leukotriene receptor antagonist, LABA = long acting beta agonist, MVI = multivitamin

[#] Correlation was determined with Spearman's rank correlation coefficient for total steroid dose in the last row.