



Correlation of vitamin D with Foxp3 induction and steroid-sparing effect of immunotherapy in asthmatic children

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ARTICLE INFO

Article history:

Received for publication April 12, 2012.

Received in revised form July 23, 2012.

Accepted for publication August 2, 2012.

ABSTRACT

Background: Vitamin D promotes different toleragenic processes of the immune system; however, its role in allergen specific immunotherapy (SIT) is still undefined.

Objective: To determine whether the immunologic and clinical effectiveness of allergen SIT depends on the serum level of 25-hydroxyvitamin D (25[OH]D) and its changes during SIT in asthmatic children.

Methods: This is a retrospective secondary analysis of pooled data obtained from our 2 recently published prospective, randomized, placebo-controlled trials on asthmatic children undergoing allergen immunotherapy. Both trials, which assessed the effect of different pharmacologic modulation of SIT effectiveness, were conducted according to the same study protocol. Children from the placebo arms in these trials were treated with immunotherapy only and were included in the present analysis. The study population consisted of 36 children. Data concerning clinical (asthma symptoms score and percent change in minimal daily inhaled corticosteroid dose) and immunologic parameters (including serum level of 25[OH]D) were analyzed in all patients.

Results: Patients with a higher serum level of 25(OH)D experienced more significant reduction in asthma symptoms score and steroid-sparing effect of SIT and had higher transforming growth factor α production and higher Foxp3 induction during SIT. Steroid-sparing effect correlated with 25(OH)D serum level at baseline, after 3 months of SIT, and with the changes in serum level of 25(OH)D during the build-up phase of SIT. Better response to SIT was observed among children with an 25(OH)D serum level higher than 30 ng/mL.

Conclusion: The efficacy of allergen SIT correlates with 25(OH)D serum concentration. It seems that a serum level of 25(OH)D higher than 30 ng/mL facilitates the optimal effect of allergen immunotherapy.

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Introduction

The discovery that many cells express vitamin D receptors and the recognition of widespread vitamin D insufficiency have stimulated interest in the potential role of vitamin D in nonskeletal conditions.¹ Serum levels of vitamin D correlate with pulmonary function,² asthma onset,³ and the development of allergic diseases.^{4,5} However, these data in adults are less convincing than in children.⁶ There is an increasing evidence to support the role of the vitamin D pathway in the regulation of the function of both innate and adaptive immune systems.⁷ Interestingly, vitamin D promotes toleragenic dendritic cells, leading to the induction of Foxp3⁺ regulatory cells⁸ and to the inhibition of both T_H1 and T_H2 responses in

a dose-dependent manner.⁹ On the basis of clinical and observational data, the plasma level of 25-hydroxyvitamin D (25[OH]D) may serve as a marker to detect or define a subclinical deficiency of vitamin D.¹⁰

Allergen specific immunotherapy (SIT) is the only available potentially curative approach in the management of allergic diseases. Therapies that modify immune regulation by enhancing T-regulatory cell activity during SIT might further enhance its effectiveness.¹¹ Natural activity of vitamin D seems to be highly attractive in the context of the mechanism and clinical effect of SIT.¹² We have previously reported the positive effect of vitamin D supplementation on the reduction of asthma symptoms and inhaled corticosteroid (ICS) dose in asthmatic children during allergen SIT.¹³ Interestingly, such a clinical effect was accompanied by higher induction of Foxp3⁺ cells and an increase in transforming growth factor β 1 (TGF- β 1)-positive cultured peripheral blood mononuclear cells after 12 months of SIT.¹³

The hypothesis of a potential relationship between the serum level of 25(OH)D and the effect of allergen immunotherapy was

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Disclosures: Authors have nothing to disclose.

Funding Sources: This study was funded by grant 503-2056-1 from the Medical University of Lodz, Lodz, Poland.

originally of interest.¹³ However, this study was underpowered to effectively perform a correlation analysis between vitamin D and ICS dose reduction during SIT.¹³ Currently, we decided to aggregate the data from our 2 different studies, conducted with the same protocol, and implement comprehensive, post hoc correlation analysis to determine whether immunologic and clinical effectiveness of SIT depends on serum level of 25(OH)D in asthmatic children.

Methods

This is a retrospective secondary analysis of pooled data obtained from our 2 recently published prospective, randomized, placebo-controlled trials involving asthmatic children undergoing allergen immunotherapy.^{13,14} Both trials, which assessed the effect of different pharmacologic modulation of SIT effectiveness, were conducted at the same time, in the same allergy center, and according to the same study protocol. Children from the placebo arms in these trials were treated with immunotherapy only and were included in the present analysis. In both studies, immunotherapy was maintained for at least 12 months in all placebo children. Dropouts and children included in per-protocol populations had a similar effect of the intervention (50%–75% reduction in the ICS dose); therefore, all analyses were conducted in the intent-to-treat population ($n = 36$). The study population consisted of 36 boys and girls aged 6 to 12 years (18 participants from each trial). All children had moderate IgE-dependent asthma with a duration of at least 2 years who were treated with ICSs with a daily dose between 400 and 800 μg (data from medical records). Patients were sensitized only to house dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), as evidenced by a positive skin prick test result (12 tested allergens in total) and by the presence of serum specific IgE. Asthma was stable in all patients; there had been no exacerbations of the disease and no change in maintenance medications or symptoms for 6 months. Characteristics of patients before immunotherapy are given in Table 1.

Study design of the included trials

The patients attended 1 prestudy and 3 study visits at the clinic. During the prestudy visit (at the beginning of a run-in period from September 2005 until the end of March 2006), all participants were informed about the purpose of the study, were told how to score asthma symptoms, and underwent skin testing. During this period, ICS dose was adjusted stepwise to define the lowest dose necessary to control asthma symptoms (according to clinical examination and lung function measurement). During the first visit, all patients

received the first subcutaneous dose of commercial *D pteronyssinus* (50%) and *D farinae* (50%) extracts (Phostal, Stallergenes, France). The second visit took place 3 months after the first visit, at the end of the build-up phase of SIT. The last study visit took place at the end of the first year of SIT. During each study visit, blood samples were obtained from all patients, clinical symptoms were assessed, lung function was measured, and the patients' diaries were evaluated. The visits were scheduled in such a way that blood sampling and lung function testing in individual patients always took place at the same time and on the same day of each week. During the prestudy visit, antiallergen measures¹⁵ were recommended for all patients. All children remained in the same environment during the study. In addition, 3-month visits were scheduled to adjust the corticosteroid doses to the current control of asthma symptoms, according to clinical examination and lung function measurement. Immunotherapy was continued throughout the study period according to a standard dose schedule (maintenance dose was 0.8 mL, which corresponds to 5.9 μg of major allergens). The average cumulative dose for each patient was 93 IR, corresponding to 686 μg of major allergens.

Vitamin D measurement

Serum level of 25(OH)D was assessed twice: at the first visit and then on the second visit (the build-up phase of SIT). Data obtained from this assessment were published only in one of the trials.¹³ Serum 25(OH)D₃ was measured by radioimmunoassay (Biosource, Camarillo, California) according to the manufacturer's instructions.

Assessment of the immunologic effects of SIT

Peripheral blood mononuclear cells (PBMCs) isolated from whole blood by density gradient centrifugation on Histopaque-1077 were cultured for 72 hours in standard mammalian cell culture conditions (37°C, 5% carbon dioxide, 80% relative humidity) in the presence of Derp2 allergen at a concentration of 10 $\mu\text{g}/\text{mL}$. CD4, CD25, and Foxp3 expression on cultured PBMCs was estimated by flow cytometry (LSRII instrument; BD Biosciences, San Jose, California) using appropriate fluorophore-conjugated monoclonal antibodies. The interleukin (IL) 10, IL-13, and TGF- β 1 levels in cell culture supernatants were determined using enzyme-linked immunosorbent assay–based specific tests (R&D, Minneapolis, Minnesota) according to the manufacturer's instructions. Foxp3 complementary DNA (cDNA) quantitation was performed by a real-time polymerase chain reaction run on a LightCycler 480 thermocycler (Roche Applied Science, Indianapolis, Indiana) using LightCycler 480 SYBR Green I Master (Roche Applied Science) according to the manufacturer's protocol. The number of FOXP3 cDNA molecules in a sample was expressed in relation to the number of hypoxanthine phosphoribosyltransferase (HPRT1) cDNA molecules, with *HPRT1* being the reference gene of choice for lymphoid cells, using the relative quantitation feature of LightCycler 480 software. The *HPRT1*-specific primers used were 5'-TGACACTG-GCAAAACAATGCA-3' and 5'-GGTCCTTTTACCAGCAAGCT-3' (sequence taken from identification No. 5 in the real-time primer database RTPrimerDB). The *FOXP3*-specific primers used were 5'-AACAGCACATTCCCAGAGTTCCT-3' and 5'-CATTGAGTGTCCTGC-TTCT-3' (sequence taken from identification No. 3792 in the real-time primer database RTPrimerDB).

Assessment of the clinical effects of SIT

The clinical effects of SIT were assessed via each patient's diary card,¹⁶ lung function measurements,¹⁷ and analysis of the reduction in the ICS dose. Asthma exacerbations were noted at each visit at the clinic. Because the monitoring of the consumption of ICSs seems to be the best approach for assessing the clinical efficacy of SIT in asthmatic children,¹⁸ the inhaled steroid-sparing effect of SIT

Table 1
Patient characteristics before immunotherapy

Characteristic	Study group ($N = 36$)
Age, No. (%)	
6–8 years	12 (33)
9–10 years	12 (33)
11–12 years	12 (33)
Male, No. (%)	11 (61)
Duration of asthma, median (IQR), y	3 (2–6)
Asthma symptom score, mean (SD) ^a	0.28 (0.03)
FEV ₁ , % of predicted values, mean (SD)	93.3 (7)
ICS dose, No. (%) ^b	
400 $\mu\text{g}/\text{d}$ of budesonide	13 (36)
600 $\mu\text{g}/\text{d}$ of budesonide	14 (39)
800 $\mu\text{g}/\text{d}$ of budesonide	9 (25)

Abbreviations: FEV₁, forced expiratory volume in 1 second; ICS, inhaled corticosteroid; IQR, interquartile range.

^aMean daily score calculated at baseline (data from September 2005 to the end of March 2006).

^bMinimal daily ICS dose capable of maintaining asthma control.

Table 2
Partial correlations among the study variables^a

	Minimal daily ICS dose, % change	Asthma symptom score, change after 12 m-s	25(OH)D, baseline	25(OH)D, after 3 m-s	25(OH)D, change after 3 m-s
Relative expression of Foxp3, change from baseline after 3 m-s	-0.27	-0.39 ^b	0.19	0.23	0.24
Relative expression of Foxp3, change from baseline after 12 m-s	-0.398 ^b	-0.38 ^b	0.34 ^b	0.16	0.02
%CD4 ⁺ CD25 ⁺ Foxp3 ⁺ , change from baseline after 3 m-s	0.15	0.15	-0.01	-0.31	-0.22
%CD4 ⁺ CD25 ⁺ Foxp3 ⁺ , change from baseline after 12 m-s	-0.487 ^c	-0.33 ^b	0.39 ^b	0.49 ^c	0.27
IL-10, change from baseline after 3 m-s	0.03	0.11	0.17	-0.11	-0.11
IL-10, change from baseline after 12 m-s	0.12	0.21	-0.04	-0.12	0.04
TGF- β 1, change from baseline after 3 m-s	-0.429 ^b	-0.33	0.33	0.23	0.02
TGF- β 1, change from baseline after 12 m-s	0.03	-0.03	-0.09	-0.02	0.01
IL-13, change from baseline after 3 m-s	0.02	0	-0.10	-0.23	-0.09
IL-13, change from baseline after 12 m-s	0.24	0.23	-0.32	-0.26	0.05
25(OH)D, baseline	-0.48 ^c	-0.50 ^c		0.65 ^d	-0.09
25(OH)D, after 3 m-s	-0.64 ^d	-0.67 ^d	0.65 ^d		0.61 ^d
25(OH)D, change from baseline after 3 m-s	-0.50 ^c	-0.39 ^b	-0.09	0.61 ^d	

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; IL, interleukin; TGF- β 1, transforming growth factor β 1.

^aAll correlations between 2 different variables were controlled for the effects of other variables given in the table. Data presented as correlation coefficient.

^b $P < .05$.

^c $P < .01$.

^d $P < .001$.

was considered the main clinical outcome of this study. A diary card was completed during the run-in period (September through March: the season with the maximum levels of natural allergen exposure),¹⁹ the build-up phase (April through June: outside the peak exposure season), and the last 7 months of the maintenance phase (September through March: the season with the maximum levels of natural allergen exposure). For these periods the mean asthma symptom score was calculated.

Diary card

The daily diary card included daytime symptoms (completed at bedtime), incidents of nocturnal awakening (recorded in the morning on awakening), and the number of puffs of as-needed β_2 -agonist. The daytime asthma symptoms and nocturnal awakenings were scored subjectively as follows: 0, no symptoms during the day or night; 1, symptoms that did not affect any activities during the day or night; 2, symptoms that affected at least one daily activity or disturbing the night; and 3, symptoms that affected 2 or more daily activities or sleep disturbances throughout all night or most of the night. The use of β_2 -agonists was scored as follows: 0, none; 1, once a day; 2, 2 to 3 times a day; and 3, more than 3 times a day. The minimum score for each day was 0 (no symptoms during the daytime, no symptoms at night, and no use of β_2 -agonists). The maximum score was 9 (severe symptoms during the day and night and the use of the β_2 -agonists >3 times a day). The daily symptoms score was provided by children and caregivers. Adherence was monitored during each SIT visit by the specialist in the context of clinical symptoms and results of lung function.

Principles for reducing the ICS dose

All patients were treated with budesonide (dry powder inhalers) (Pulmicort Turbuhaler; AstraZeneca, London, United Kingdom) twice daily. Before the first visit (the run-in period) and after the second visit (the maintenance phase of SIT) the daily dose of ICS was reduced stepwise every 3 months until asthma was no longer controlled. The treatment was then continued with a dose one step higher. The dose steps of budesonide were 100, 200, 400, 600, and 800 μ g daily. When the patient was asymptomatic for 3 months with the minimal dose, ICS treatment was discontinued. Uncontrolled asthma was defined according to guidelines²⁰ based on

daytime and nighttime asthma symptoms, lung function, and the number of short-acting β_2 -agonist inhalations. Reducing the ICS dose was not allowed during the build-up phase of SIT. The daily dose of ICS was assessed during the entire study. On the basis of the minimal daily dose capable of maintaining asthma control during the September through March period before SIT and during the SIT period, the reduction in the minimal daily dose of ICS after the first year of SIT was calculated for all patients. Reduction in ICS dose was calculated according to the following rule: (ICS dose after the first year of SIT – ICS dose at baseline)/ICS dose at baseline $\times$ 100%.

Statistical analyses

The associations of the range of immunologic and clinical parameters with 25(OH)D level were assessed by partial correlation analysis. The partial correlations procedure computes correlation coefficients that describe the linear relationship between 2 variables while controlling for the effects of other additional variables. Such an approach allowed us to assess the relationship between vitamin D serum level and the clinical effect of SIT independently from the potential effect of other variables (immunologic markers of SIT).

To determine differences between the groups, Mann-Whitney or Fisher exact tests were used. To estimate the cutoff level of vitamin D for the prediction of a better response to SIT receiver operating characteristic curve analysis was implemented. All statistical analyses were performed using StatSoft Statistica for Windows, release 8.0 (StatSoft Inc, Tulsa, Oklahoma). $P < .05$ was used as a definition of statistical significance.

Results

Correlations

We conducted the correlation analysis of clinical and immunologic outcomes obtained during 12 months of SIT in 36 asthmatic children. The results of all correlation analyses are given in Table 2. All correlations between 2 different variables were controlled for the effects of other variables given in the table.

After 12 months of SIT, the median daily ICS dose necessary to control the symptoms of asthma was reduced by 50% (Fig 1B). Significant negative correlations between the serum level of

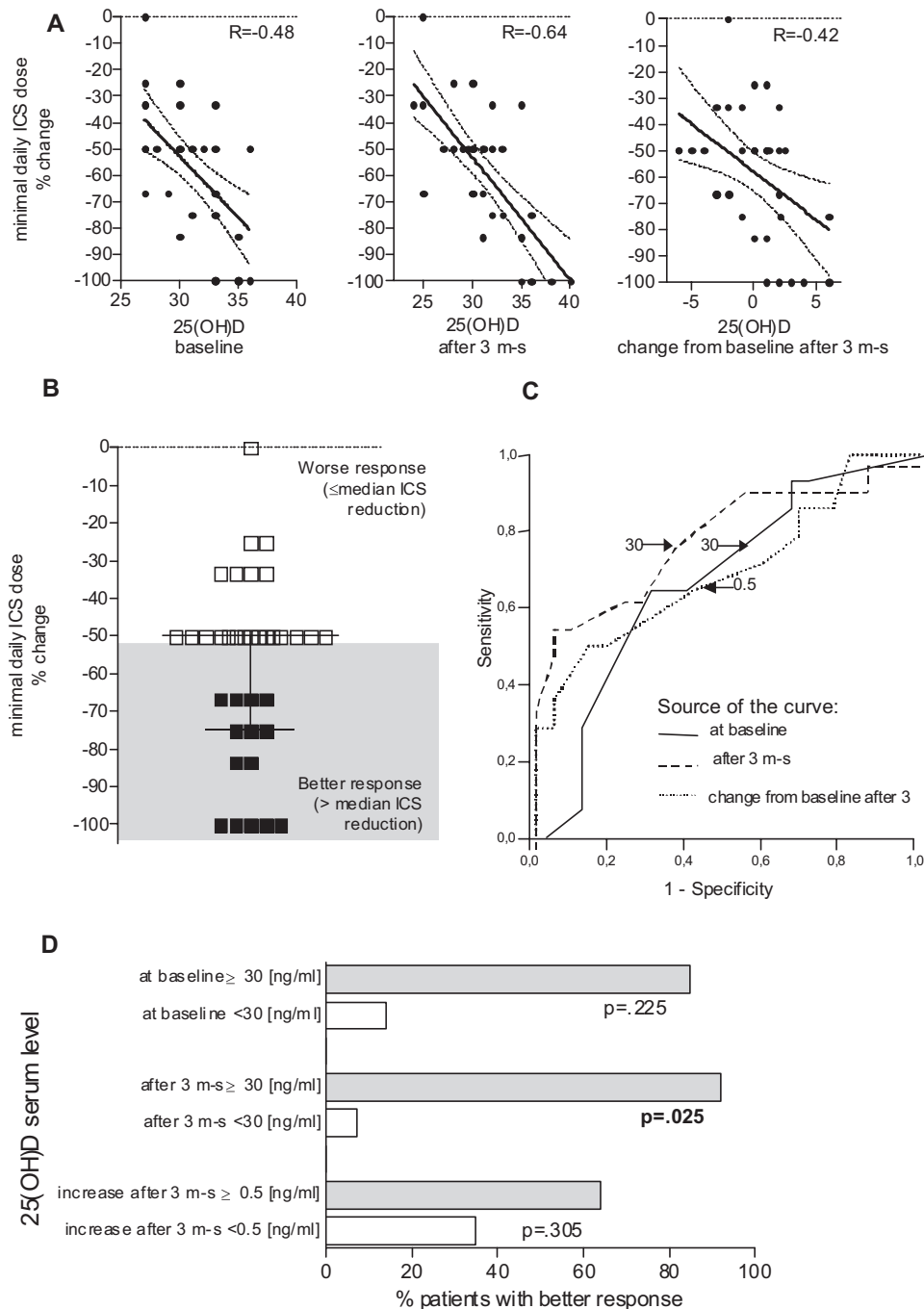


Fig. 1. A, Linear correlations between the serum level of 25-hydroxyvitamin D (25[OH]D) and the reduction of the minimal daily dose of inhaled corticosteroid (ICS) necessary to control the symptoms of asthma (percent change). B, The reduction of the minimal daily dose of ICS necessary to control the symptoms of asthma (percent change) in each participant (a better response to specific immunotherapy [SIT] defined as $>50\%$ ICS dose is indicated as dark squares). C, Receiver operating characteristic curve for serum level of 25(OH)D in the prediction of a better response to SIT. D, Percentage of participants with a better response to SIT according to the serum level of 25(OH)D. Reduction in ICS dose was calculated according to the following rule: (ICS dose after the first year of SIT – ICS dose at baseline)/ICS dose at baseline $\times 100\%$. Better response to SIT was defined as more than median (50%) reduction in minimal daily ICS dose.

25(OH)D (measured at baseline and after 3 months and as a change from baseline) and the minimal daily ICS dose reduction were found (Fig 1A). We also observed significant negative correlations between percent change in the minimal daily ICS dose and changes from baseline after 12 months of SIT in the relative expression of Foxp3 and the percentage of Foxp3⁺ cells (interpreted as a reduction in the minimal controlling dose of ICS is associated with an increase in Foxp3 during SIT). Minimal daily ICS dose negatively correlated also with TGF- β production after 3 months of SIT. Patients with higher TGF- β production, higher Foxp3 induction, and

higher 25(OH)D serum concentration experienced more significant steroid-sparing effect of SIT. Similar correlations were observed with asthma symptom scores. In addition, the correlation between messenger RNA (mRNA) relative expression in Foxp3 induced after 3 months of SIT and the reduction of asthma symptoms score after 12 months of SIT was observed.

Correlation analyses were conducted between serum level of 25(OH)D and immunologic outcomes. Baseline 25(OH)D serum concentration correlated positively with TGF- β 1 production in the PBMC cultures of patients after 3 months of SIT and with Foxp3

Table 3
Sensitivity, specificity, PPV, and NPV, of 25(OH)D in the prediction of a more significant steroid-sparing effect of allergen immunotherapy (greater than the median value of ICS dose reduction)

25(OH)D	ICS dose reduction		Sensitivity	Specificity	PPV	NPV
	Less than or equal to median value	Greater than median value				
Baseline						
≤30 ng/mL	8	2	85.7	36.4	46.1	80
>30 ng/mL	14	12				
After 3 m-s			92.8	45.4	52	90.9
≤30 ng/mL	10	1				
>30 ng/mL	12	13				
Change from baseline after 3 m-s			64.3	59.1	50	72.2
≤0.5 ng/mL	13	5				
>0.5 ng/mL	9	9				

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ICS, inhaled corticosteroid; NPV, negative predictive value; PPV, positive predictive value.

induction after 12 months of SIT observed as mRNA expression and the percentage of Foxp3-positive cells. Serum level of 25(OH)D measured after 3 months of SIT correlated with the percentage of Foxp3-positive cells measured after 12 months of SIT. No significant correlation was found at baseline between 25(OH)D serum concentration and the minimal daily dose of ICS controlling asthma symptoms.

Prediction value of vitamin D serum level

In the next step, reduction in minimal daily ICS dose was dichotomized in the subgroups according to the following rules: (1) less than or equal median value (50%) defined as a worse response to SIT and (2) more than median value defined as a better response to SIT (Fig 1B). Minimal daily ICS dose reduction dichotomized as described together with the serum level of 25(OH)D were included in the receiver operating characteristic curve analysis (Fig 1C). The cutoff levels of 25(OH)D serum concentration with the highest sensitivity and specificity to predict the better response to SIT, measured at baseline and after 3 months of SIT and as a change from baseline after 3 months of SIT, were calculated as follows: 30, 30, and 0.5 ng/mL. We observed a higher percentage of patients with better response to SIT among those with a 25(OH)D serum level greater than 30 ng/mL measured after 3 months of SIT (Fig 1D). The sensitivity, specificity, positive predictive value, and negative predictive value of better response to SIT for these cutoff levels of 25(OH)D were calculated and are given in Table 3.

Finally, all immunologic outcomes were compared among the steroid-sparing subgroups. Better response to SIT was observed in patients with a higher serum level of 25(OH)D, higher Foxp3 induction, and higher production of TGF- β 1 (Fig 2).

Discussion

We found that clinical and immunologic efficacy of allergen SIT in children with asthma allergic to house dust mites was correlated with the 25(OH)D serum concentration. Patients with a higher serum level of 25(OH)D experienced more significant reduction in asthma symptoms score and corticosteroid-sparing effect of SIT, had higher TGF- β production, and higher Foxp3 induction during 12 months of SIT.

Our group recently published a study demonstrating the effect of vitamin D supplementation on early efficacy of immunotherapy.¹³ We observed that combined administration of a systemic corticosteroid and allergen extract suppressed early clinical and immunologic effects of SIT and that vitamin D₃ prevented this adverse effect of corticosteroids. Despite the controversies concerning the route of the administration, timing, and doses of vitamin D applied in that study, a favorable effect of vitamin D in immunotherapy is encouraging.²¹ In this study, we found that asthma symptoms score and ICS dose reduction after 12 months of SIT positively correlate with

the serum level of vitamin D measured at baseline and at 3 months of SIT. A clinical effect of blood levels of vitamin D on asthma control was previously observed independently from SIT. For example, it has recently been demonstrated that lower levels of vitamin D are associated with reduced asthma control.²² These results indicate that hypovitaminosis D is frequent in children with asthma living in Mediterranean countries. In those children, lower levels of vitamin D are associated with reduced asthma control. In our study, improved asthma control and ICS dose reduction observed during SIT were correlated with the serum level of vitamin D. Our study also did not use a control group with asthma who did not receive SIT. Although we were able to demonstrate correlations between vitamin D levels and outcomes, such as symptom scores, ICS dose controlling the symptoms, TGF- β production, and FoxP3 induction, one could conclude that these effects are related to vitamin D levels independent of SIT. However, we also found significant correlation between changes in asthma control and minimal ICS dose with vitamin D serum level during SIT; therefore, it seems fair to conclude that vitamin D modulates SIT efficacy.

Vitamin D has been shown to have several effects on the innate and adaptive immune system that might modulate the response to allergen immunotherapy.^{7,9,13,23} Vitamin D, a potent inhibitor of nuclear factor- κ B expression, has potentially beneficial effects on the adaptive immune system through its effects on dendritic cell maturation and T-regulatory cells induction.²² Vitamin D (alone or with glucocorticoids) has been reported to promote differentiation of naive T cell into IL-10-secreting T-regulatory cells.^{10,13} Vitamin D has also been shown to increase serum levels of the immunomodulatory cytokines TGF- β and IL-10 in humans^{9,12,24} and to enhance the benefits of allergen immunotherapy in a murine model by IL-10- and TGF- β -dependent mechanisms.²⁵ In our study, we found that the serum level of 25(OH)D correlates with an early immunologic response measured by TGF- β 1 production in the PBMC cultures of patients after 3 months of SIT. Importantly, 25(OH)D serum level also correlates with Foxp3 induction assessed with mRNA expression and by percentage of Foxp3⁺ cells among peripheral CD4⁺CD25⁺. However, on the basis of our results, we can only speculate that vitamin D may boost the immunologic effect of SIT via modulation of the cytokine microenvironment or direct modulation of Foxp3 cell induction.

These observations raise several clinical questions. First, keeping in mind that vitamin D serum levels depend on sunlight exposure, should the season of the year be included when planning the start of SIT? Second, because we observed an inverse correlation between 25(OH)D serum level and body mass index among our patients, should we suspect a worse response to SIT in obese children? Finally, should vitamin D be supplemented in patients with 25(OH)D insufficiency before SIT?

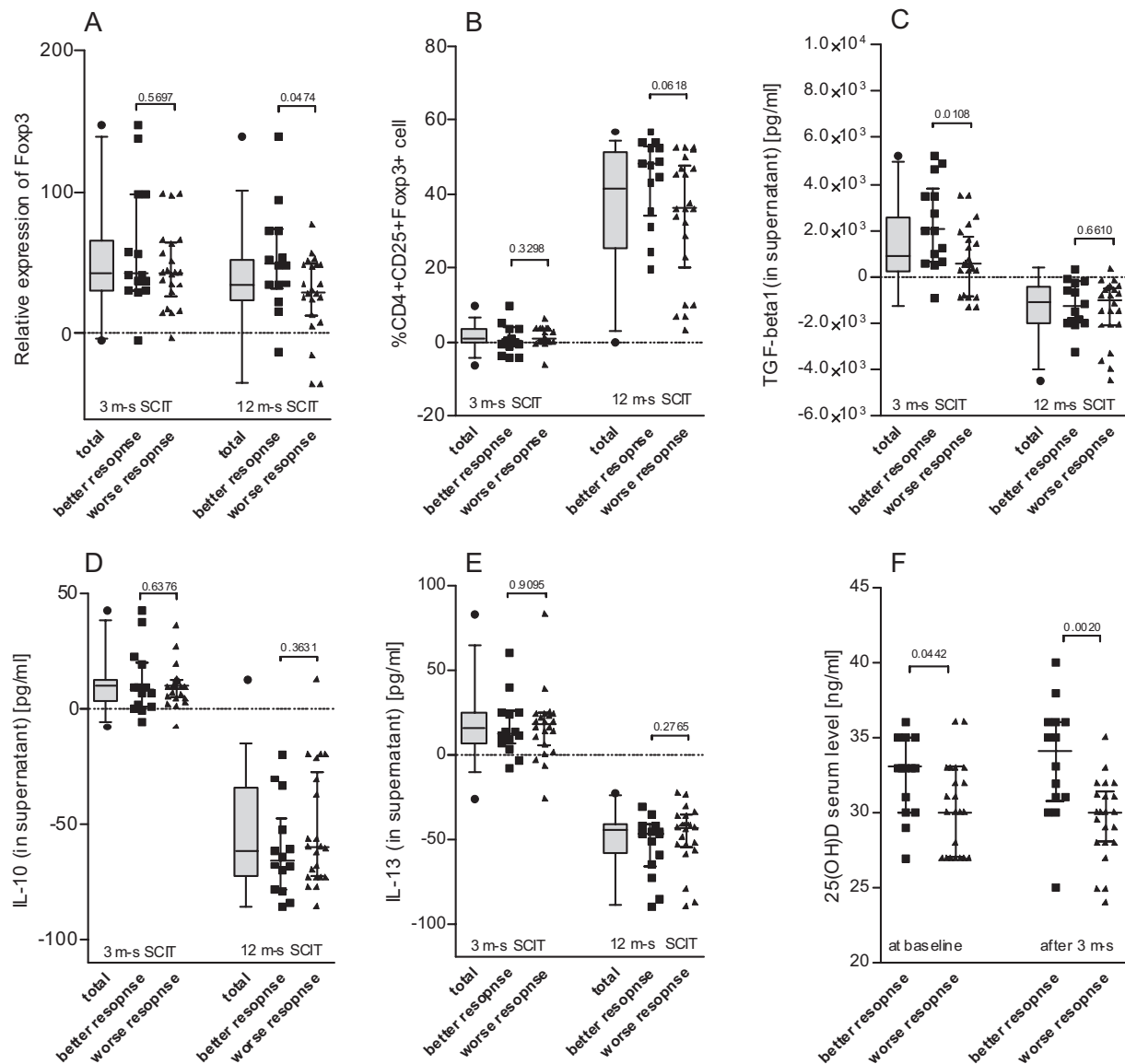


Fig. 2. Immunologic parameters presented as change from baseline after 3 (3 m-s SCIT) and 12 months (12 m-s SCIT) of specific immunotherapy (SIT) in the total ($n = 36$) group and in the 2 subgroups of patients with a worse (defined as less than median [50%] reduction in minimal daily inhaled corticosteroid [ICS] dose) and better (defined as more than median [50%] reduction in minimal daily ICS dose) response to SIT. SCIT indicate subcutaneous immunotherapy; square or triangles, median values for each patient; error bars, inter quartile ranges. The following parameters are presented in the panel: relative expression of Foxp3 (A), %CD4⁺CD25⁺Foxp3⁺ cell (B), transforming growth factor β 1 (TGF- β 1) (C), interleukin (IL) 10 (D), IL-13 (E), and 25-hydroxyvitamin D (25[OH]D) (F). TGF- β 1, IL-10, and IL-13 were measured in supernatant of PBMC allergen-specific stimulation; 25(OH)D was measured in serum. For between-group comparisons, Mann-Whitney tests were used. Reduction in ICS dose was calculated according to the following rule: (ICS dose after the first year of SIT – ICS dose at baseline)/ICS dose at baseline $\times$ 100%.

In the next step of our analysis, to predict a better response to SIT in our population, a cutoff level of 25(OH)D serum concentration at 30 ng/mL was estimated. What is noteworthy is that our estimated level is equal to the recommended serum level of 25(OH)D in children.²⁶ We observed a lower percentage of patients with a better response to SIT among those with an insufficient (<30 ng/mL) 25(OH)D serum level measured after 3 months of SIT. What is more, we observed that relatively small change in vitamin D correlates with ICS dose reduction during SIT. It may raise the question concerning clinical significance of such a finding. It could be explained by the fact that in most patients the 25(OH)D serum level was stable. Nevertheless, our results suggest that the kinetics of vitamin D serum level are important during SIT in the context of its clinical efficacy.

In the previous studies, clinical parameters, such as age, asthma duration and severity, breadth of allergic sensitization, and ongoing

natural allergen exposure, were proposed as predictors of steroid-sparing effect of SIT.²⁷ In the present study, we found that the steroid-sparing effect of SIT is associated with TGF- β production and CD4⁺CD25⁺Foxp3⁺ cell induction. In our opinion, the vitamin D observation is of greater clinical value than the other associations because it opens new possibilities for pharmacologic modulation of SIT effectiveness. Moreover, we observed that steroid-sparing effect correlates with vitamin D serum level at baseline, after 3 months of SIT, and with the changes in the serum level of vitamin D during the build-up phase of SIT. These results support the role of vitamin D in allergen immunotherapy of IgE-dependent asthma.

Our study has some limitations. This is a retrospective study based on correlation analyses in a relatively small sample size. Our results do not allow use to assess the cause-and-effect relationship between vitamin D and SIT effectiveness; therefore, conclusions should be verified in a well-designed randomized control trial.

Because we found that the efficacy of allergen SIT correlates with 25(OH)D serum concentration, we recommend monitoring the serum level of 25(OH)D in children undergoing allergen immunotherapy, especially in those at risk of vitamin D insufficiency. It seems that a serum level of 25(OH)D above 30 ng/mL facilitates the optimal effect of allergen immunotherapy.

Acknowledgment

We are indebted to Stallergenes Pharmaceutical Company for providing extracts for SIT and Der p 2 for in vitro stimulation.

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