Vitamin D reduces eosinophilic airway inflammation in nonatopic asthma

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Background: Low levels of vitamin D are associated with asthma severity, airway remodeling, and exacerbation rate increase, especially in nonatopic asthma. Reduced steroid responsiveness or impaired antimicrobial defense might be underlying mechanisms.

Objective: We sought to evaluate the effect of vitamin D supplementation on eosinophilic and neutrophilic airway inflammation in patients with nonatopic asthma.

Methods: In a double-blind, randomized, placebo-controlled trial, we investigated the effect of long-acting vitamin D_3 (400,000 IU) on sputum neutrophils and eosinophils in 44 patients with nonatopic asthma with neutrophilic (≥53%) and/or eosinophilic (≥3%) airway inflammation. Sputum induction was performed at baseline and after 9 weeks. Other measurements included questionnaires, blood samples, and pulmonary function.

Results: Treatment with vitamin D did not significantly affect sputum neutrophils or eosinophils compared with treatment with placebo in the total group. Regarding sputum eosinophils, the effect of vitamin D appeared to be dependent on baseline sputum eosinophil levels (interaction $P = .015$). In patients with eosinophil levels of 26.2% or more (median in patients with sputum eosinophilia, >3%), eosinophils decreased from a median of 41.0% to 11.8% after vitamin D treatment as compared with an increase from 51.8% to 63.3% in patients receiving placebo ($P = .034$). Vitamin D treatment also resulted in slightly better Asthma Control Questionnaire scores ($P = .08$).

Conclusions: Vitamin D supplementation reduced eosinophilic airway inflammation in patients with nonatopic asthma with severe eosinophilic airway inflammation, but did not affect sputum neutrophils. Also, a small effect on asthma control was observed. These findings suggest that vitamin D might have potential as an add-on treatment option in eosinophilic asthma.

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Key words: *Asthma, vitamin D, airway inflammation, eosinophils, neutrophils, nonatopic*

There is a growing body of evidence suggesting an association between asthma and vitamin D. Low levels of vitamin D have been related to poor asthma control,[1] more hospitalizations for asthma,[2] and a higher incidence of respiratory tract infections.[3] Moreover, in children, supplementation of vitamin D has been shown to be effective in reducing the incidence of asthma exacerbations[4] and respiratory tract infections.[5]

Remarkably, recent studies showed that the association between vitamin D insufficiency and asthma exacerbations was stronger in patients without atopy than in subjects with atopy.[6,7] Nonatopic asthma is a common, but relatively underexposed asthma phenotype.[8] Epidemiological evidence has shown that this type of asthma is associated with adult onset of disease,[9] more severe symptoms,[10] faster decline in FEV_1,[11] and higher socioeconomic costs.[12] The factors triggering nonatopic asthma are not always clear. Several studies suggest that particularly in patients without atopy with an adult onset of their disease, respiratory tract infections play a central role.[13,14] It has been hypothesized that in these patients, colonization with specific pathogens might induce neutrophilic airway inflammation,[15] but it has also been speculated that microbial superantigens are the unknown triggering factor in nonatopic asthma, increasing T_H2 cells with infiltration of eosinophils.[16]

Although the underlying mechanisms are not yet known, the beneficial effects of vitamin D might be attributed to its anti-inflammatory functions. On the one hand, it may reduce neutrophilic inflammation by its ability to reduce neutrophil chemotaxis[17] and by boosting the immune defense against microorganisms.[18] On the other hand, vitamin D may reduce eosinophilic airway inflammation by enhancing corticosteroid responsiveness.[19] Therefore, we hypothesized that supplementation of vitamin D improves asthma control by reducing eosinophilic and/or neutrophilic airway inflammation, particularly in nonatopic asthma. To test this hypothesis, we investigated the effect of oral vitamin D_3 preparation (cholecalciferole) on eosinophil and neutrophil counts in induced sputum in patients with nonatopic asthma. In addition, the effects on asthma control,

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Abbreviations used
FENO: Fraction of nitric oxide in exhaled air
FVC: Forced vital capacity

asthma-related quality of life, nasal symptoms, exhaled nitric oxide, pulmonary function, and eosinophil and neutrophil counts in peripheral blood were evaluated.

METHODS

Patients

Patients with asthma aged 18 years or older were included in this study. Asthma diagnosis was confirmed by a documented reversible airway obstruction (improvement in FEV_1 ≥12% predicted and ≥200 mL after the administration of 400 μg of salbutamol) or by airway hyperresponsiveness to methacholine (reduction of 20% predicted in FEV_1 after the inhalation of up to 8 mg/mL of methacholine). All patients were using standard asthma medication according to international guidelines.[20] They were all nonatopic (no allergic symptoms at any time and absence of specific IgE to common inhalation allergens and fungi) and they had neutrophilic (induced sputum neutrophils ≥53%[21]) and/or eosinophilic (induced sputum eosinophils ≥3%[22]) airway inflammation. Patients had not experienced exacerbations or acute respiratory tract infections in the 4 weeks before enrolment. Smokers and ex-smokers were allowed to participate in the study provided they had at least 12% predicted reversibility in FEV_1 and a normal diffusion capacity of CO (transfer factor of the lung for CO, ≥80% of predicted) at the time of inclusion. Patients with vitamin D levels of more than 100 nmol/L at baseline were excluded to lower the risk of causing hypercalcemia. Further exclusion criteria were other pulmonary comorbidity (eg, sarcoidosis and bronchiectasis), contraindications for vitamin D use (eg, history of kidney stones), previous use of high-dose supplementary vitamin D, hypercalcemia, and pregnancy. The local medical ethical board approved the study. Written informed consent was obtained from every patient before participation in the study. This study was registered in the Dutch trial register (NTR2205).

Study design and treatment

This randomized, double-blind, placebo-controlled single-center study was conducted in the pulmonary outpatient clinic of a general hospital in Leeuwarden, The Netherlands. Patients were recruited from a large cohort study of patients with nonatopic asthma. The study consisted of 3 visits: 1 visit at baseline, 1 visit at 1 week after taking study medication to check vitamin D_3 plasma levels, and 1 visit 9 weeks after taking study medication to evaluate the effect of vitamin D_3 on primary and secondary outcome measures. At baseline (visit 1), patients completed questionnaires, underwent spirometry, and had blood samples taken, levels of exhaled nitric oxide (FENO) assessed, and sputum induced. Patients with confirmed sputum eosinophilia (≥3%) and/or neutrophilia (≥53%) were randomly assigned to receive either a single high dose of long-acting oral vitamin D_3 preparation (400,000 IU cholecalciferole, De Collegiale bereiding, Oldenzaal, The Netherlands) or placebo. The study medication was packaged uniformly by a clinical pharmacist and later added to yogurt, which was finished by the patient under the supervision of a blinded, independent investigator.

Patients continued their normal asthma medication and were instructed not to use other vitamin supplements. If asthma medication (except short-acting beta-agonists) was changed, patients were excluded from the study. After 1 week (visit 2), blood samples were taken to measure vitamin D_3 levels. Also, adverse events were assessed using a questionnaire asking for symptoms (headache, abdominal complaints, dysuria, etc) and blood calcium level was measured. At visit 3 (9 weeks after inclusion), all assessments of visits 1 and 2 were repeated.

Outcome measures

Primary outcomes. The 2 primary outcomes were the changes from baseline in neutrophil and eosinophil counts in induced sputum at 9 weeks after vitamin D_3 administration. Sputum was induced using a standardized protocol,[23] and whole sputum samples were processed. Differential cell counts were calculated as a percentage of nonsquamous cells. Sputum samples were eligible for analysis if they contained less than 80% squamous epithelial cells.

Secondary outcomes. Secondary outcomes included changes in Asthma Quality of Life Questionnaire score (range, 1-7[24]), Asthma Control Questionnaire score (range, 0-6[25]), Sino-Nasal Outcome Test score (range, 0-110[26]), peripheral blood eosinophil and neutrophil counts, total IgE, FEV_1, forced vital capacity (FVC), FEV_1/FVC ratio, and FENO.

FEV_1 and FVC were measured according to standard procedures[27] before and 30 minutes after the administration of 400 μg of salbutamol, and FENO was measured by using a handheld NO analyzer (Niox Mino, Accuramed, Nossegem, The Netherlands).[28]

Measurement of vitamin D_3. Plasma 25-hydroxyvitamin-D_3 was quantified using liquid chromatography and isotope dilution tandem mass spectrometry using a reagent kit from Chromsystems (Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany).

Statistical analysis

Between-group differences at baseline were investigated by using 2-sample *t* tests or Mann-Whitney *U* tests for continuous data and the Fisher exact or the χ^2 test for categorical data, whenever appropriate. Changes in parameters were calculated as percentages of baseline values. Differences in changes in parameters between both groups were analyzed using Mann-Whitney *U* tests. Analysis of covariance was used to evaluate the effect of baseline levels of vitamin D, as well as baseline percentages of sputum eosinophils and neutrophils, on the effect of treatment. The relationship between changes in vitamin D_3 levels and changes in sputum eosinophils and neutrophils was investigated using Spearman rank correlation coefficient.

For patients with neutrophilic airway inflammation, group sample sizes of 12 and 12 achieve 80% power to detect a difference of 30.0 units (%) between the mean change in percentage of neutrophils in induced sputum in the vitamin D group compared with the mean change in percentage of neutrophils in the placebo group, with SDs of 25.0 (%) and with a significance level (α) of .05 using a 2-sided 2-sample *t* test. For the effect of vitamin D treatment on the change in eosinophils in sputum, we expected a similar effect size as for the neutrophils.

A 2-tailed *P* value of less than .05 was considered to indicate statistical significance. All analyses were performed using SAS software, version 9.2 (SAS Institute, Inc, Cary, NC).

RESULTS

Of the 196 patients who were screened for this study, 118 had a successful sputum induction. Of these subjects, 44 patients were eligible for participation in the study (15 patients with sputum eosinophilia and 20 with sputum neutrophilia and 9 with mixed eosinophilia and neutrophilia) and data from these patients were used in the analysis (Fig 1). Baseline characteristics showed no statistically significant differences between patients treated with vitamin D and patients treated placebo (Table I). For the total group, baseline characteristics are presented in more detail in Table E1 in this article's Online Repository available at www.jacionline.org.

Vitamin D_3 measurements

At baseline, plasma vitamin D_3 levels were low (<50 nmol/L) in 15 patients (6 patients in the vitamin D group and 9 patients in the placebo group; $P = .26$). One patient in each group was deficient (vitamin D_3 < 30nmol/L). Vitamin D_3 levels showed a

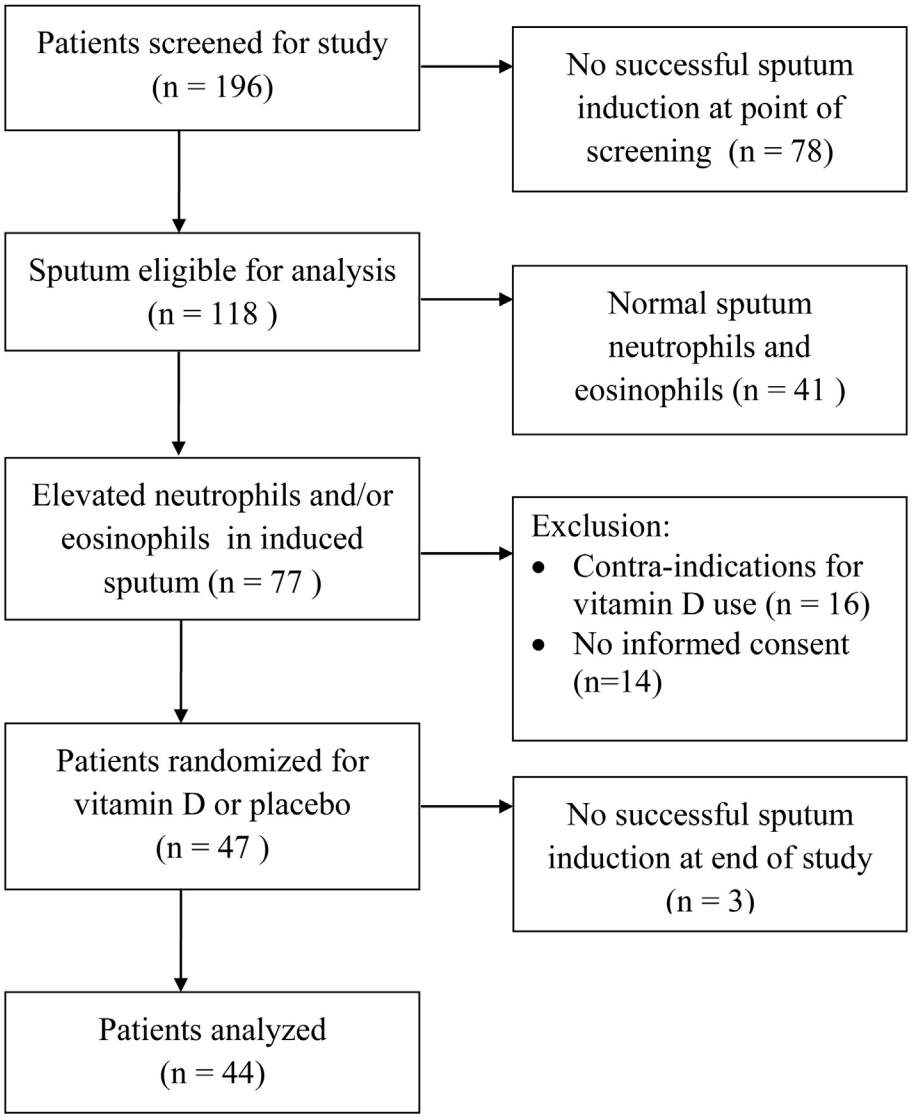

FIG 1. Flowchart describing the inclusion and exclusion of patients.

significant rise from the median level of 60 nmol/L to 153 nmol/L ($P < .01$) after 1 week and 91 nmol/L after 9 weeks ($P < .01$) in the treated group.

Effect of vitamin D_3 on sputum neutrophils

The percentage of sputum neutrophils showed a slight, nonsignificant reduction in the placebo group and no change in the vitamin D group (Table II). This change in sputum neutrophil percentage was not significantly different between both groups. The change in plasma vitamin D_3 levels was not related to the change in percentage sputum neutrophils. No interaction was observed between baseline vitamin D level or sputum neutrophil percentage and the treatment effect on sputum neutrophils.

Effect of vitamin D on sputum eosinophils

Sputum eosinophils showed no change in the vitamin D group as compared with the placebo group ($P = .69$), with the median percentage change from baseline of the sputum eosinophils being −0.12% (95% CI, −17.7 to 35.1) in the placebo group and −0.17% (95% CI, −31.2 to 49.6) in the vitamin D group. However, when evaluating the impact of baseline values on treatment effect, a significant interaction between the 2 parameters was observed (interaction $P = .015$), indicating modification of the effect of vitamin D_3 dependent on the baseline sputum eosinophil level.

Within the group of patients with sputum eosinophilia, those with the highest percentages of eosinophils (≥26.2%, based on the median of the group, "high eosinophil group") showed a substantial decrease in sputum eosinophils from a median of 41.1% to 11.8% in the vitamin D group (median percentage change, −65.8%; 95% CI, −101.2 to −12.9; $P = .034$), as compared with an increase from 51.8% to 63.3% in the placebo group (median percentage change, −3.5%; 95% CI, −38.5 to 84.3; $P = .65$) (Fig 2). In these patients in the high eosinophil group, changes in plasma vitamin D_3 levels were correlated with changes in the percentage of sputum eosinophils

TABLE I. Baseline characteristics

Characteristic	Placebo (n = 22)	Vitamin D (n = 22)	*P* value
Sex: male (%)	68.2	50	.22
Age (y)*	53.6 ± 16.7	59.0 ± 9.7	.20
Adult onset (%)	86.4	81.8	.68
BMI (kg/m^2)*	26.9 ± 4.8	26.6 ± 4.2	.86
Asthma duration (y)†	5 (2-16)	4 (3-30)	.79
Pack-years†	6 (0-21)	2 (0-11)	.20
ICS dose, budesonide equivalent†	400 (400-400)	400 (400-800)	.26
AQLQ score†	5.7 (5.2-6.3)	6.0 (5.1-6.4)	.41
ACQ score†	1.2 (0.7-1.6)	0.9 (0.4-1.9)	.70
SNOT score†	22 (18-43)	23 (14-30)	.53
Vitamin D_3 (nmol/L)†	57 (40-70)	60 (47-78)	.43
Blood eosinophils (10^9/L)†	0.2 (0.1-0.4)	0.2 (0.1-0.3)	.72
Blood neutrophils (10^9/L)†	3.8 (2.9-4.5)	3.4 (2.7-4.5)	.98
IgE (kU/L)†	69 (1-2110)	29 (13-117)	.81
pbFEV_1 (%pred)*	97.6 ± 18.1	99.1 ± 15.7	.76
pbFEV_1/FVC (%pred)*	89.4 ± 12.8	92.5 ± 11.4	.41
FENO (ppb)†	33 (15-67)	24 (19-36)	.35
Sputum neutrophils (%)†	67.8 (33.8-75.6)	64.8 (44.6-76.4)	.15
Sputum eosinophils (%)†	6.7 (0.2-39.7)	3.2 (0.3-13.3)	.50
Sputum eosinophils ≥3%, n (%) of patients	12 (54.5)	12 (54.5)	1
Sputum neutrophils ≥53%, n (%) of patients	13 (59.1)	16 (72.7)	.34

ACQ, Asthma Control Questionnaire; *AQLQ*, Asthma related Quality of Life Questionnaire; *BMI*, body mass index; *ICS*, inhaled corticosteroid; *pb*, postbronchodilator; *ppb*, parts per billion; *%pred*, percentage of predicted value; *SNOT*, Sino-Nasal Outcome Test.
*Mean ± SD.
†Median (first and third interquartiles).

($r = -0.72$; $P = .01$). No correlations were observed between changes in vitamin D_3 levels and changes in FEV_1 ($r = 0.15$; $P = .33$), FEV_1/FVC ($r = -0.22$; $P = .16$), Asthma Control Questionnaire score ($r = -0.11$; $P = .49$), and Asthma related Quality of Life Questionnaire score ($r = .08$; $P = .60$). In the patients in the "low to moderate eosinophil group" (sputum eosinophils <26.2%), no effect of vitamin D_3 was found.

The effect of vitamin D_3 on secondary parameters

Secondary outcomes are listed in Table II. We observed a trend toward better asthma control as reflected in a slight reduction in Asthma Control Questionnaire score in the vitamin D group as compared with the placebo group ($P = .08$). There were no significant changes in Asthma related Quality of Life Questionnaire and Sino-Nasal Outcome Test scores or peripheral blood eosinophils, neutrophils, and total IgE. There was a significant difference in the change in FEV_1/FVC ratio between both groups, which was explained by a reduction in the patients receiving placebo.

Adverse events

Hypercalcemia was not observed in any of the patients and calcium levels did not change after supplementation with vitamin D_3. The total number of adverse events was similar in both groups (7 vs 9 patients in the placebo group vs the vitamin D group; $P = .80$). No differences were observed in adverse events derived from the questionnaire.

DISCUSSION

In this study, a single high dose of oral vitamin D_3 reduced eosinophilic airway inflammation in patients with nonatopic asthma and prominent sputum eosinophilia. Vitamin D_3 did not affect sputum neutrophils despite a significant rise in plasma vitamin D_3 levels. These results suggest that the beneficial effect of vitamin D_3 in asthma may be related to its effect on eosinophilic airway inflammation, thereby identifying an area of potential intervention in the subgroup of patients with most severe asthma.

To our knowledge, this is the first study evaluating the effect of vitamin D_3 as an add-on treatment on airway inflammation in adult patients with asthma. So far, intervention studies with vitamin D in asthma have mainly been performed in children. Also, most studies have focused on clinical outcomes, without paying attention to the underlying mechanisms. In schoolchildren, vitamin D supplementation has been shown to reduce seasonal influenza A episodes and asthma attacks[5] and the addition of vitamin D to inhaled budesonide in steroid-naive asthmatic children has been shown to reduce the exacerbation rate.[4] Our finding of vitamin D_3 affecting eosinophilic airway inflammation might provide a possible mechanistic explanation for these latter results. In adults, there are not many studies regarding the effect of vitamin D on exacerbations. Although no effect on exacerbations was seen in a recent study, a small reduction in the maintenance dose of inhaled corticosteroids was observed.[29] This could be in line with our finding of a reduction in sputum eosinophils after vitamin D supplementation. Regarding infections, vitamin D supplementation in adults has been shown to have little effect on the incidence of upper respiratory tract infections.[30-32] Data on vitamin D supplementation in asthma are limited, and our study adds to the field by showing that vitamin D_3 reduces eosinophilic airway inflammation in adults with severe airway inflammation.

In the subgroup of patients with eosinophilic airway inflammation, we observed a significant lowering effect of vitamin D supplementation on sputum eosinophils, but not on FENO levels. These divergent effects on sputum eosinophils and FENO have been observed in earlier studies as well, suggesting that sputum eosinophils and FENO are biomarkers of different pathophysiological mechanisms.[33]

The strength of our study is the use of a single high dose of oral vitamin D_3 that significantly increased plasma vitamin D_3 levels, even after a 9-week interval, in contrast to other intervention studies in which no increase was found or measured. With this strategy, we were able to exclude possible nonadherence with vitamin D_3 treatment. We chose a single high dose of oral vitamin D_3 (400,000 IU) on the basis of experimental studies in which vitamin D doses of up to 400,000 IU had been proven to be safe.[34] Also, in our study, no adverse events from treatment were reported. With this high dose of vitamin D_3, there was no effect on neutrophilic airway inflammation and it seems unlikely that higher doses or other dosing regimens would have led to different results. This lack of effect is also unlikely to be related to the small sample size because even a trend of an effect on sputum neutrophils was not observed. With respect to sputum eosinophils, we cannot exclude that similar reductions could have been obtained with lower doses of vitamin D.

The lowering effect of vitamin D_3 on sputum eosinophils might be explained by its effect on steroid sensitivity. *In vitro* studies

TABLE II. Effect of vitamin D on airway inflammation and secondary outcomes

	Placebo (n = 22)			Vitamin D (n = 22)			
Outcome	**Baseline**	**9 wk**	***P* value**	**Baseline**	**9 wk**	***P* value**	**Between-group *P* value**
Primary outcomes							
Sputum eosinophils (%)	6.7 (0.2-39.7)	3.9 (0.2-50.9)	.86	3.1 (0.3-13.3)	0.7 (0.2-11.4)	.21	.69
Sputum neutrophils (%)	67.8 (33.8-75.6)	50.5 (22.5-72.1)	.36	64.8 (44.6-76.4)	65.5 (45.1-86.2)	.58	.30
Secondary outcomes							
AQLQ score, total score	5.7 (5.2-6.3)	6.0 (5.6-6.2)	.26	6.0 (5.1-6.4)	6.3 (6.0-6.6)	.007	.47
AQLQ score, symptoms	5.5 (4.8-6.1)	5.8 (5.2-6.3)	.29	5.7 (5.0-6.3)	6.2 (5.7-6.5)	.001	.10
AQLQ score, activities	5.9 (4.9-6.4)	5.9 (5.3-6.3)	.97	6.0 (5.3-6.7)	6.3 (6.0-6.7)	.033	.21
AQLQ score, emotional	6.0 (5.6-6.5)	6.1 (5.8-6.6)	.16	6.6 (5.8-6.8)	6.6 (6.4-6.8)	.28	.70
AQLQ score, environmental	5.4 (4.9-6.5)	5.8 (4.7-6.3)	.65	5.9 (4.7-6.3)	6.1 (5.5-6.6)	.077	.10
ACQ score	1.2 (0.7-1.6)	1.1 (0.8-1.6)	.53	0.9 (0.4-1.9)	0.8 (0.4-1.3)	.064	.08
SNOT score	22 (18-43)	17 (12-32)	.076	23 (14-30)	14 (10-31)	.043	.82
Calcium (mmol/L)	2.36 (2.31-2.41)	2.34 (2.28-2.37)	.018	2.39 (2.33-2.45)	2.38 (2.30-2.44)	.10	.47
Vitamin D (nmol/L)	57 (40-70)	48 (39-65)	.28	60 (47-78)	91 (82-106)	<.001	<.001
Blood eosinophils (10^9/L)	0.2 (0.1-0.4)	0.2 (0.1-0.4)	.49	0.2 (0.1-0.3)	0.2 (0.1-0.3)	.50	.71
Blood neutrophils (10^9/L)	3.8 (2.9-4.5)	3.6 (3.0-4.9)	.71	3.4 (2.7-4.5)	3.5 (2.4-4.6)	.18	.19
IgE (kU/L)	69 (5-265)	47 (4-264)	.50	29 (13-117)	29 (13-88)	.30	.49
$pbFEV_1$ (%pred)*	97.6 ± 18.1	94.0 ± 17.1	.039	99.1 ± 15.7	97.4 ± 15.7	.27	.44
$pbFEV_1$/FVC (%pred)*	89.4 ± 12.8	86.0 ± 11.3	.005	92.5 ± 11.4	92.5 ± 11.5	.92	.03
FENO (ppb)	33 (15-67)	26 (11-60)	.30	24 (19-36)	22 (17-29)	.23	.52

ACQ, Asthma Control Questionnaire; *AQLQ*, Asthma related Quality of Life Questionnaire; *pb*, postbronchodilator; *ppb*, parts per billion; *%pred*, percentage of predicted value; *SNOT*, Sino-Nasal Outcome Test.
*Mean ± SD; all other values are presented as median (first and third interquartiles).

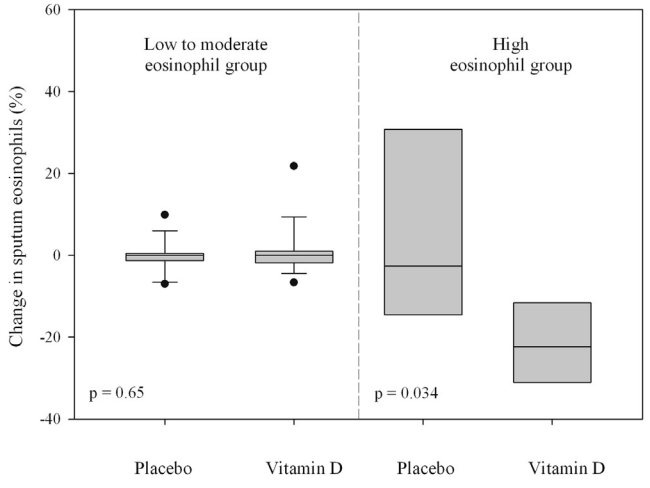

FIG 2. Effect of vitamin D_3 on eosinophilic airway inflammation. Box plots of the change from baseline in percentage sputum eosinophils after placebo or vitamin D_3 treatment in the "low to moderate eosinophil group" (sputum eosinophils <26.2%; *left panel*) and the "high eosinophil group" (sputum eosinophils ≥26.2%; *right panel*). For placebo and vitamin D_3, the number of patients was 14 and 18, respectively, in the low to moderate eosinophil group and 8 and 4, respectively, in the high eosinophil group. Medians, 25th and 75th percentiles, and 95% CIs are presented. The *thick black lines* in the boxes represent the medians of the change in percentage sputum eosinophils, the bottoms and tops of the boxes represent the first and third quartiles of the distributions of the change in percentage sputum eosinophils, and the stacks represent the 2.5th and 97.5th percentiles of the distributions. The *P* values are comparing the distributions of the change in percentage sputum eosinophils of placebo or vitamin D group patients, separately in the subgroups of patients with low/moderate or high baseline sputum eosinophils.

showed that vitamin D_3 restores the IL-10 response,[35] supporting the idea that vitamin D improves steroid responsiveness[19] and reduces eosinophilic airway inflammation. This is supported by several clinical studies. An analysis of the Childhood Asthma Management Program data set showed that higher serum vitamin D levels were associated with better response to inhaled corticosteroid therapy.[36] Other studies showed a relationship between lower levels of vitamin D and the requirement for higher doses of inhaled corticosteroid in children[37-39] and a decreased response to glucocorticoids in adults.[40] Our results confirm and extend these findings and suggest that vitamin D treatment might be particularly beneficial in the subgroup of patients with asthma with eosinophilic airway inflammation.

The lack of effect of vitamin D_3 on neutrophilic airway inflammation was surprising because previous findings suggested that low levels of vitamin D_3 might induce neutrophilic airway inflammation. In addition to a direct immune modulatory effect, as illustrated by its ability to reduce the production of the neutrophil chemoattractant IL-8 by lung epithelial cells,[17] vitamin D is also thought to enhance innate immunity against microorganisms by inducing the production of antimicrobial peptides.[18] This suggests that low levels of vitamin D could lead to ongoing neutrophilic inflammation by reduced clearance of infectious agents and lower suppression of inflammatory responses. In contrast to these previous findings, we did not observe any effect of vitamin D_3 on sputum neutrophils.

The lack of efficacy of vitamin D_3 on neutrophilic airway inflammation might be due to the inclusion of patients without vitamin D deficiency. We purposely included patients regardless of their vitamin D_3 level because the current normal values of plasma vitamin D_3 apply to patients with diseases other than asthma, such as osteoporosis. Nevertheless, when comparing the effects of vitamin D_3 versus placebo on inflammatory cells between the groups with and without vitamin D insufficiency (vitamin D <50 nmol/L), we observed no differences. Another reason for a lack of effect of vitamin D_3 on neutrophilic inflammation could have been the selection of patients with relatively mild asthma. It is conceivable that this selection affected the results of this study. Therefore, it cannot be excluded that vitamin D_3 would

have reduced neutrophilic airway inflammation in patients with more severe asthma.

In conclusion, our results suggest that vitamin D_3 as an add-on treatment might be able to reduce eosinophilic airway inflammation in patients with nonatopic asthma with high levels of sputum eosinophils, possibly by enhancing steroid responsiveness. Vitamin D_3, with its favorable safety profile and low costs, might be a promising steroid-sparing agent in subgroups of patients with asthma with persistent airway eosinophilia.

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Key messages

- **Vitamin D might reduce sputum eosinophils in patients with nonatopic asthma and prominent sputum eosinophilia.**
- **Vitamin D has no affect on sputum neutrophilia in these patients.**

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TABLE E1. Baseline characteristics of total group (n = 44)

Characteristic	Mean ± SD	Minimum	First interquartile	Median	Third interquartile	Maximum
Age (y)	56.3 ± 13.8	18	48	60	67	73
Age of onset (y)	43.8 ± 20.3	2	29	48	60	71
Asthma duration (y)	12.6 ± 14.8	1	2	5	22	56
Pack-years	9.9 ± 15.1	0	0	3	15	80
ICS dose, budesonide equivalent	507 ± 378	0	400	400	800	1600
OCS dose (mg/d)	0.6 ± 2.2	0	0	0	0	0-10
BMI (kg/m^2)	26.8 ± 4.4	19.2	23.9	26.0	29.4	38.1
AQLQ score	5.7 ± 0.74	3.3	5.1	5.9	6.3	6.7
ACQ score	1.2 ± 0.70	0.1	0.5	1.1	1.7	3.1
SNOT score	26 ± 14.9	3	16	23	33	66
Vitamin D_3 (nmol/L)	59 ± 21.1	27	41	57	73	113
Blood leukocytes (10^9/L)	6.7 ± 1.9	3.5	5.6	6.2	7.4	13.7
Blood eosinophils (10^9/L)	0.2 ± 0.19	0	0.1	0.2	0.3	0.8
Blood neutrophils (10^9/L)	3.7 ± 1.5	1.4	2.8	3.7	4.5	9.6
IgE (kU/L)	160 ± 352	1	12	43	183	2110
pbFEV_1 (%pred)	98.3 ± 16.8	45	89	100	112	110
pbFEV_1/FVC (%pred)	90.9 ± 12.1	54	84	91	101	110
FENO (ppb)	40 ± 35.7	7	16	30	48	182
Sputum neutrophils (%)	59.0 ± 24.5	10.0	42.1	65.3	75.8	98.0
Sputum eosinophils (%)	15.7 ± 22.4	0	0.2	3.7	26.9	80.8

ACQ, Asthma Control Questionnaire; *AQLQ*, Asthma related Quality of Life Questionnaire; *BMI*, body mass index; *ICS*, inhaled corticosteroid; *OCS*, oral corticosteroid; *pb*, postbronchodilator; *ppb*, parts per billion; *%pred*, percentage of predicted value; *SNOT*, Sino-Nasal Outcome Test.

TABLE E2. Baseline characteristics of patients with high sputum eosinophils and low sputum eosinophils at baseline

Characteristic	Low sputum eosinophils (n = 32)	High sputum eosinophils (n = 12)	*P* value
Sex: male (%)	53.1	75.0	.19
Age (y)*	53.2 ± 13.2	64.7 ± 12.0	.01
Adult onset (%)	84.4	83.3	.93
BMI (kg/m^2)*	27.1 ± 4.8	26.0 ± 3.4	.47
Asthma duration (y)†	4 (3-22)	5 (2-19)	.79
Pack-years†	2 (0-15)	3 (1-31)	.28
ICS dose, budesonide equivalent†	400 (400-800)	400 (400-400)	.71
AQLQ score†	5.9 (5.0-6.3)	5.9 (5.4-6.3)	.41
ACQ score†	1.0 (0.4-1.7)	1.4 (0.7-1.9)	.44
SNOT score†	27 (13-41)	20 (18-23)	.20
Vitamin D_3 (nmol/L)†	58 (40-75)	57 (44-66)	.59
Blood eosinophils (10^9/L)†	0.2 (0.0-0.2)	0.4 (0.3-0.6)	<.001
Blood neutrophils (10^9L)†	3.6 (2.7-4.6)	3.8 (2.9-4.2)	.97
IgE (kU/L)†	17 (5-66)	210 (75-312)	<.001
pbFEV$_1$ (%pred)*	100.3 ± 14.1	93.1 ± 22.3	.21
pbFEV$_1$/FVC (%pred)*	93.9 ± 10.6	83.0 ± 12.6	.01
FENO (ppb)†	22 (13-34)	79 (41-111)	<.001
Sputum neutrophils (%)†	71.5 (61.4-77.9)	27.0 (14.9-45.2)	<.001
Sputum eosinophils (%)†	0.4 (0.2-6.8)	42.1 (34.3-61.6)	<.001
Sputum eosinophils ≥3%, n (%) of patients	12 (37.5)	12 (100)	<.001
Sputum neutrophils ≥53%, n (%) of patients	28 (87.5)	1 (8.3)	<.001

ACQ, Asthma Control Questionnaire; *AQLQ*, Asthma related Quality of Life Questionnaire; *BMI*, body mass index; *ICS*, inhaled corticosteroid; *OCS*, oral corticosteroid; *pb*, postbronchodilator; *ppb*, parts per billion; *%pred*, percentage of predicted value; *SNOT*, Sino-Nasal Outcome Test.

*Mean ± SD.

†Median (first and third interquartiles).

TABLE E3. Individual data of patients receiving vitamin D_3 or placebo in the high eosinophil group

Patient (n = 12)	Treatment group	Sputum eosinophils at baseline	Sputum eosinophils at 9 wk
1	Placebo	26.4	8.6
2	Vitamin D	27.0	8.8
3	Placebo	32.8	87.4
4	Placebo	38.6	70.5
5	Vitamin D	40.8	8.2
6	Vitamin D	41.3	14.8
7	Placebo	42.8	42.2
8	Vitamin D	56.4	47.0
9	Placebo	60.8	56.0
10	Placebo	61.8	89.4
11	Placebo	68.4	49.2
12	Placebo	80.8	76.2

Table shows individual data of all patients in the high eosinophil group. Values of sputum eosinophils as a percentage of the total nonsquamous cell counts are shown at baseline and 9 wk after treatment.