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. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

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