Low Serum Vitamin D During Remission Increases Risk of Clinical Relapse in Patients With Ulcerative Colitis

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BACKGROUND & AIMS: **Vitamin D levels have been associated with disease activity in patients with ulcerative colitis (UC), but it is unclear whether they affect the risk of disease relapse. We sought to determine the association between baseline vitamin D levels during a period of clinical remission and risk of subsequent UC relapse.**

METHODS: **We performed a physician-blinded prospective study of 70 patients with UC in clinical remission followed up after a surveillance colonoscopy at a tertiary academic medical center. Serum samples were collected at the time of colonoscopy and baseline endoscopic and histologic activity were determined. Levels of 25-hydroxy-vitamin D were measured using an enzyme-linked immunosorbent assay. The primary outcome was rate of clinical relapse, determined over 12 months.**

RESULTS: **The mean baseline vitamin D level was lower among patients with relapse (29.5 ng/mL) than without (50.3 ng/mL) ($P = .001$). Remission vitamin D level (≤35 ng/mL) was associated with a risk of clinical relapse (odds ratio, 1.25; 95% confidence interval [CI], 1.01–1.56; $P = .044$) over 12 months, independent of endoscopic or histologic grade at enrollment. A receiver operating characteristic curve of vitamin D levels for the outcome of relapse had an area under the curve of 0.72; and a serum level of 35 ng/mL or less had a sensitivity of 70% (95% CI, 46%–88%) and a specificity of 74% (95% CI 57%–83%) for predicting risk of clinical relapse.**

CONCLUSIONS: **Serum levels of vitamin D of 35 ng/mL or less during periods of clinical remission increase the risk of UC relapse. Clinical trials to obtain vitamin D levels higher than this threshold should be considered.**

Keywords: IBD; Biomarker; Relapse Prevention.

See editorial on page 247.

Ulcerative colitis (UC) and Crohn's disease are inflammatory bowel diseases (IBDs) that are chronic, idiopathic disorders of the gastrointestinal tract that are believed to result from a complex interplay between genetic, environmental, immune, and microbial factors.[1,2] Vitamin D has an emerging role as an environmental factor in the pathogenesis of autoimmune disorders including IBD.[3,4] The clinical significance of vitamin D has extended beyond its function in calcium homeostasis and bone metabolism, with mounting evidence supporting its role in regulating immune responses.[5,6] Vitamin D receptors are found to be expressed on a variety of immune cells and indeed vitamin D has numerous and multifaceted effects on the immune system. Vitamin D decreases the generation of proinflammatory T-helper 1 responses and increases the levels of anti-inflammatory T-helper 2 cells.[7] Vitamin D promotes self-tolerance by inhibition of dendritic cell differentiation and maturation[8,9] and increases the number and function of T-regulatory cells.[10,11] Vitamin D also has been implicated in modulating the ability of human macrophages to kill intracellular bacteria[12] as well as protecting the epithelial mucosal barrier in intestinal inflammation.[13]

Genetic polymorphisms in the vitamin D receptor (VDR) have been associated with susceptibility and disease severity in patients with ulcerative colitis and Crohn's disease.[14,15] In mice models of experimental

Abbreviations used in this paper: **CI, confidence interval; IBD, inflammatory bowel disease; OR, odds ratio; SCCAI, Simple Colitis Clinical Activity Index; UC, ulcerative colitis; VDR, vitamin D receptor.**

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colitis, VDR knockout mice developed severe inflammation of the gastrointestinal tract and administration of the active form of vitamin D (1,25D3) attenuated this effect of VDR deficiency on the development of severe colitis.[16] In human studies, vitamin D levels are related inversely to disease severity in patients with IBD. In 2 cross-sectional studies, vitamin D deficiency was associated with disease activity in both ulcerative colitis and Crohn's disease.[17,18] One retrospective study showed the association between vitamin D deficiency and lower quality-of-life indices and increased disease activity in patients with Crohn's disease, but not in patients with UC.[19] Several other studies in Crohn's disease patients have shown that higher vitamin D status was associated with a reduced risk of disease development, normalization of vitamin D levels reduced risk of surgery, and that vitamin D levels increase and are responsive to treatment with biologics.[20–22]

Prospective studies exploring the effects of vitamin D on clinical outcomes and relapse are lacking, especially in patients with ulcerative colitis. One small prospective cohort of patients with mild to moderate Crohn's disease showed that increasing the serum vitamin D levels to normal levels was associated with decreased disease index scores.[23] In a randomized clinical trial of vitamin D supplementation in Crohn's disease patients, there was a trend toward a reduced risk of clinical relapse, but ultimately was not significant.[24] Although vitamin D status has been implicated to correlate with disease severity, the clinical significance of low vitamin D levels among patients with ulcerative colitis in clinical remission is unclear. We thus aimed to explore the effect of vitamin D levels on risk of relapse in patients with UC in clinical remission. We also assessed the correlation between vitamin D levels and the presence of baseline endoscopic and histologic inflammation.

Materials and Methods

Study Design and Patient Enrollment

We conducted a prospective study of patients with UC in clinical remission, with a Simple Clinical Colitis Activity Index (SCCAI) of 2 or less (Supplementary Figure 1),[25,26] who were recruited after a surveillance colonoscopy from the Inflammatory Bowel Disease Center at Beth Israel Deaconess Medical Center, Harvard Medical School (Boston, MA) from 2009 to 2012. The study was approved by the Beth Israel Deaconess Medical Center Institutional Review Board under protocol 2009P000314. From a cohort of 170 enrolled subjects, 70 had a baseline serum sample collected at the time of index colonoscopy. After the study follow-up period was completed, blinded investigators (J.G. and S.M.) measured serum vitamin D levels in these de-identified samples. Age, sex, ethnicity, smoking status, creatinine level, duration of disease, extent of disease, relevant medications (current nonsteroidal anti-inflammatory drugs, current mesalamine, current 6-mercaptopurine/azathioprine, current anti–tumor necrosis factor-α, steroids in the past year, and vitamin D supplementation), and season of enrollment were recorded for each patient. Baseline laboratory values (white blood cell count, hematocrit level, erythrocyte sedimentation rate, and C-reactive protein level) also were obtained. We did not assess for baseline dietary vitamin D intake, initiation of vitamin D supplementation during the follow-up period, or actively supplement patients with vitamin D based on baseline levels.

Assessment of Baseline Endoscopic and Histologic Inflammation and Clinical Relapse

Each enrolled patient had a clinically indicated surveillance colonoscopy. During the index colonoscopy, endoscopic activity was classified using the sigmoidoscopy subscore of the Mayo activity index based on the most inflamed segment of the colon.[27] Histologic activity in all segments was classified using the Geboes score by a gastrointestinal pathologist blinded to endoscopic scores.[28] For each patient, a total Geboes score was assigned to biopsy specimens from each colonic segment and the highest score (most inflamed segment) was used as the cumulative histologic score. In our study, we defined endoscopic inflammation as a Mayo endoscopic score of 2 or greater. Histologic inflammation was defined as a Geboes histologic score of 3 or greater. Clinical relapse during the follow-up period was defined as a SCCAI score greater than 2, medication intensification, or UC-related hospitalization at any time during our follow-up period of 12 months. Medication intensification was defined by an increase in dose of the current regimen, addition of another medication, or change in class of medication as a result of symptom relapse.

Vitamin D Measurement and Receiver Operator Characteristic Curve

Serum vitamin D levels (25(OH)D) were measured by using a commercial enzyme-linked immunosorbent assay kit (Calbiotech, San Diego, CA) according to the manufacturer's instructions. A receiver operating characteristic curve of vitamin D levels for the outcome of clinical relapse was constructed. A vitamin D level threshold of 35 ng/mL or less was determined to have the greatest association for risk of clinical relapse in univariate and multivariate analyses (Supplementary Table 1), and thus was chosen as our threshold level for further analyses. We compared the risk of clinical relapse among patients with a vitamin D level of 35 ng/mL or less vs a level greater than 35 ng/mL. To account for seasonal variations in vitamin D levels in New England, the year was dichotomized to a low sunlight season (September–February) and a high sunlight season (March–August).

Sunlight season was included as a covariate in our univariate analysis for risk of clinical relapse.

Data Analysis

The rate of clinical relapse, predictive value of clinical variables on the primary outcome, odds ratio (OR) with its 95% confidence interval (CI), and *P* values were assessed using JMP 11.0 (SAS Institute, Inc, Cary, North Carolina). Dichotomous variables were analyzed for outcomes using the chi-square test or the Fisher exact test where appropriate, and continuous variables were analyzed using *t* tests if normally distributed, or the Wilcoxon test for non-normal data. Correction for multiple testing was included. All variables were analyzed initially in a univariate fashion to determine their association with clinical relapse. *P* values of factors that showed evidence of an effect on clinical relapse ($P < .05$) then were analyzed on multivariate regression analysis. Given that mucosal healing has been associated with a risk of clinical relapse in patients with ulcerative colitis,[29,30] we constructed models accounting for the individual effects of endoscopic and histologic inflammation. We also performed a subgroup analysis restricted to patients with both clinical (SCCAI $\leq$ 2) and endoscopic (Mayo endoscopic score $\leq$1) remission while accounting for the confounding effects of underlying histologic inflammation (Geboes grade $\geq$ 3). Finally, we also performed a time-to-event analysis to detect a difference in the time to clinical relapse among patients with vitamin D level of 35 ng/mL or less vs vitamin D levels greater than 35 ng/mL. For our multivariate analysis, model building was based on forward stepwise logistic regression, with a *P* value of .05 required for entry. All figures were generated using GraphPad Prism (version 5.0; GraphPad Software, Inc, La Jolla, CA).

Results

Baseline Patient Characteristics

Table 1 details the baseline phenotype of the enrolled cohort. The mean serum vitamin D level in the cohort was 44 ng/mL (standard deviation, ±29). Only 8 patients (11.4%) used steroids in the past year. Vitamin D supplementation was reported by 42 patients (60%). Among the 70 patients enrolled, endoscopic inflammation was present in 9 patients (13%), whereas histologic inflammation was present in 32 patients (46%) at baseline. A multivariate analysis of the effects of clinical variables on the presence of baseline endoscopic or histologic inflammation highlighted that a vitamin D level of 35 ng/mL or less was associated independently with an increased presence of endoscopic inflammation (OR, 1.29; 95% CI, 1.07–1.85; $P < .01$) or histologic inflammation (OR, 1.46; 95% CI, 1.13–1.88; $P = .005$) at baseline.

Table 1. Baseline Clinical Characteristics of Cohort of Ulcerative Colitis Patients (N = 70 Patients)

Clinical characteristic	Subjects, n (%)
Demographics	
Average age, *y*	48.6 (±15.2)
Female sex	45 (64.3)
Caucasian ethnicity	65 (92.9)
Smoking, current[a]	2 (2.9)
Average creatinine level, *mg/dL*	0.86 (±0.19)
Season of enrollment (low sunlight)[b]	15 (21.4)
Ulcerative colitis characteristics	
Disease duration, average years	12.0 (±13.6)
Left-sided colitis	27 (38.6)
Extensive colitis	37 (52.9)
Duration of remission ≥ 6 mo	57 (81.4)
Medications	
Current NSAIDs	7 (10)
Current mesalamine	52 (74.3)
Current 6MP/AZA	13 (18.6)
Current anti–TNF-α	4 (5.7)
Steroids in past year	8 (11.4)
Vitamin D supplement[c]	42 (60)
Baseline laboratory values, mean ± SD	
White blood cell, *K/uL*	6.7 (±2.3)
Hematocrit, %	41.0 (±5.0)
Erythrocyte sedimentation rate, *mm/h*	9.6 (±9.3)
C-reactive protein, *mg/L*	3.2 (±4.7)
Serum vitamin D level, *ng/mL*[d]	44.0 (±29)
Baseline inflammation	
Endoscopic inflammation[e]	9 (12.9)
Histologic inflammation[f]	32 (45.7)

NSAID, nonsteroidal anti-inflammatory drug; 6MP/AZA, 6-mercaptopurine/azathioprine; TNF-α, tumor necrosis factor-α.
[a]There were only 2 patients who were current smokers at the start of the study, no patients were former smokers.
[b]Low sunlight season in Massachusetts is September to February, and the high sunlight season is March to August.
[c]Includes patients who were on baseline vitamin D supplements (including a multivitamin) at time of enrollment in the study.
[d]Baseline serum vitamin D level at the time of enrollment in the study.
[e]Endoscopic inflammation was defined as a Mayo endoscopic score of 2 or higher.
[f]Histologic inflammation was defined as a Geboes histologic score of 3 or higher.

Vitamin D Levels and Receiver Operator Characteristic Curve

All patients who later relapsed had a SCCAI greater than 2 at follow-up evaluation. Clinical relapse outcomes in our cohort included the following: SCCAI greater than 2 alone (N = 5), SCCAI greater than 2 with resulting medication intensification (N = 13), and SCCAI greater than 2 with resulting medication intensification and hospitalization (N = 2). As shown in Figure 1, the mean baseline vitamin D level was lower in those who later relapsed (29.5 ng/mL), than those who did not (50.3 ng/mL; $P = .001$). A receiver operating characteristic curve of baseline serum vitamin D levels for the outcome of 12-month clinical relapse had an area under the curve of 0.72 ($P < .01$) (Figure 2). A serum vitamin D level of 35 ng/mL or less had a sensitivity of 70% (95% CI,

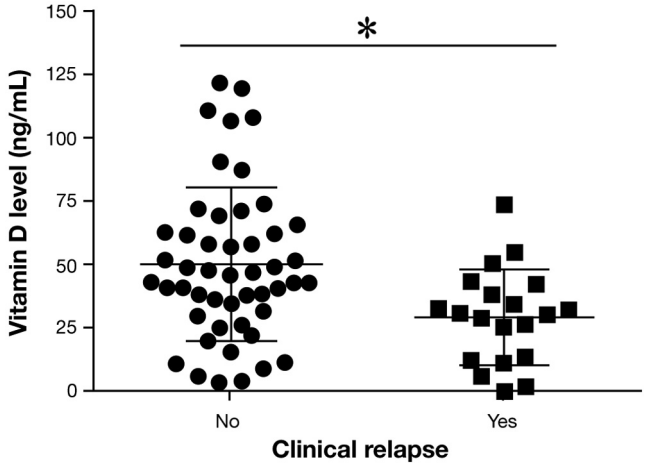

Figure 1. Distribution of serum vitamin D levels among ulcerative colitis patients by clinical relapse status at 12 months.

46%–88%) and a specificity of 74% (95% CI, 57%–83%) for predicting subsequent risk of clinical relapse. An analysis of the effect of various serum vitamin D level thresholds on risk of subsequent clinical relapse is summarized in Supplementary Table 1.

Vitamin D Threshold and Risk of Clinical Relapse

The rate of clinical relapse over 12 months for patients with a vitamin D level of 35 ng/mL or less vs patients with a vitamin D level greater than 35 ng/mL was 20% vs 9% ($P = .003$), respectively. Table 2 summarizes the univariate analysis of effect of clinical variables on clinical relapse; an increased risk of clinical relapse was associated with a vitamin D level of 35 ng/mL or less (OR, 1.26; 95% CI, 1.16–1.75; $P = .001$), steroid use in the past year (OR, 1.46; 95% CI, 1.05–2.04; $P = .026$), and histologic inflammation (OR, 1.52; 95% CI, 1.22–1.89; $P < .001$). Patients with a vitamin D level of 35 ng/mL or less also had a shorter time to clinical relapse ($P < .001$, by log-rank Mantel Cox) compared with UC patients with a vitamin D level greater than 35 ng/mL (Figure 3). In our multivariate-effects model, a baseline vitamin D level of 35 ng/mL or less was associated with an increased risk of clinical relapse (OR, 1.25; 95% CI, 1.01–1.56; $P = .044$) even after adjusting for the effects of baseline histologic inflammation (Table 3).

Table 2. Univariate Factors Associated With Clinical Relapse Over 12 Months

Clinical variables	Odds ratio	95% CI	*P* value
Age, per year	0.99	0.99–1.00	.146
Female sex	1.06	0.84–1.34	.601
Caucasian ethnicity	1.15	0.90–1.46	.257
Smoking, current	1.24	0.64–2.40	.513
Creatinine level	0.88	0.90–2.47	.107
Season, low sunlight[a]	1.22	0.98–1.51	.079
Vitamin D supplements	1.42	0.70–2.01	.244
Vitamin D level $\leq$ 35 ng/mL	1.26	1.16–1.75	.001
Current NSAIDs	1.00	0.69–1.44	.980
Current mesalamine	1.26	0.98–1.61	.073
Current 6MP/AZA	1.12	0.85–1.49	.411
Current anti–TNF-α	0.96	0.60–1.54	.859
Steroids in past year	1.46	1.05–2.04	.026
Erythrocyte sedimentation rate	1.00	0.98–1.01	.569
C-reactive protein level	0.98	0.96–1.01	.204
Endoscopic inflammation[b]	1.35	0.98–1.87	.066
Histologic inflammation[c]	1.52	1.22–1.89	<.001

NSAID, nonsteroidal anti-inflammatory drug; 6MP/AZA, 6-mercaptopurine/azathioprine; TNF-α, tumor necrosis factor-α.
[a]Low sunlight season in Massachusetts is form September to February, and high sunlight season is from March to August.
[b]Includes patients with endoscopic inflammation (Mayo endoscopic score $\geq$ 2).
[c]Includes patients with histologic inflammation (Geboes histologic score $\geq$ 3).

Discussion

In this prospective cohort study of 70 patients with ulcerative colitis, we showed that vitamin D levels are associated with baseline endoscopic and histologic inflammation severity during clinical remission, and are associated independently with the longitudinal risk of clinical relapse. These results suggest that vitamin D

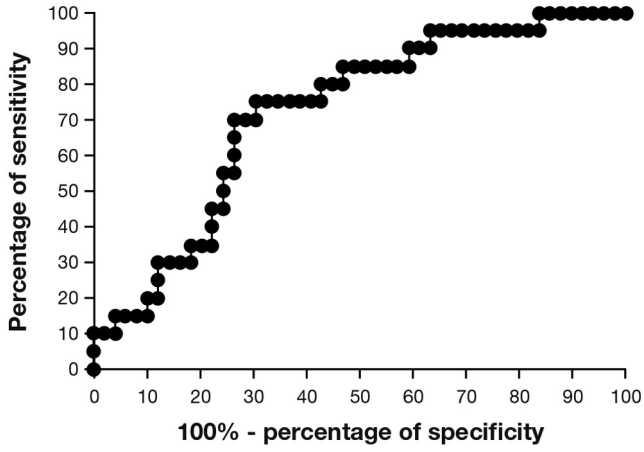

Figure 2. Receiver Operating Characteristic (ROC) curve of serum vitamin D levels for the outcome of clinical relapse.

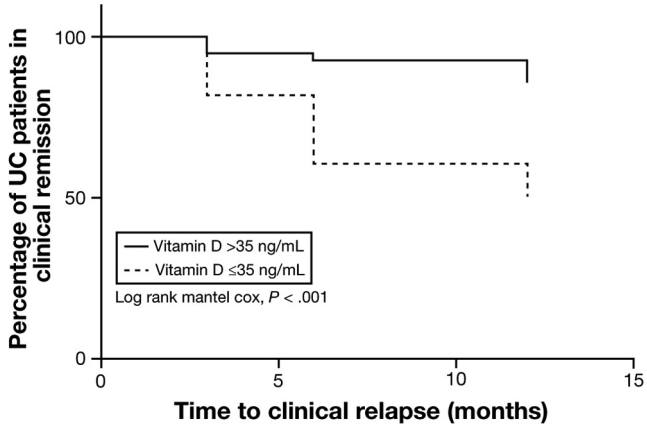

Figure 3. Time to event analysis of the effect of serum vitamin D levels on risk of clinical relapse in ulcerative colitis patients in remission.

Table 3. Multivariate Analysis of Factors Associated With Clinical Relapse Over 12 Months

Clinical variables	Odds ratio	95% CI	*P* value
Endoscopic inflammation model[a]			
Vitamin D level ≤ 35 ng/mL	1.32	1.05–1.66	.020
Steroids in past year	1.29	0.93–1.79	.131
Endoscopic inflammation	1.13	0.81–1.56	.475
Histologic inflammation model[b]			
Vitamin D level ≤ 35 ng/mL	1.27	1.03–1.57	.026
Steroids in past year	1.20	0.87–1.64	.264
Histologic inflammation	1.37	1.10–1.71	.007
Clinical and endoscopic remission model[c]			
Vitamin D level ≤ 35 ng/mL	1.25	1.01–1.56	.044
Histologic inflammation	1.40	1.13–1.74	.003

[a]Analysis included UC patients in clinical remission, model was adjusted for the confounding effects of endoscopic inflammation.
[b]Analysis included UC patients in clinical remission, model was adjusted for the confounding effects of histologic inflammation.
[c]Analysis was restricted to patients in clinical remission (SCCAI ≤ 2) and endoscopic remission (Mayo endoscopic score ≤ 1), N = 61 patients; in univariate analysis steroid use in past year was no longer associated with a risk of clinical relapse.

status is linked not only to current disease severity, but also has an impact on future risk of clinical relapse.

This was a prospective study showing the effect of vitamin D levels during remission on clinical outcomes among patients with ulcerative colitis. There have been few prospective studies exploring the role of vitamin D on ulcerative colitis clinical outcomes. One analysis of an epidemiologic cohort by Ananthakrishnan et al[21] showed that low vitamin D levels was associated with a clinical relapse in the form of an increased risk of hospitalizations and surgeries. The results of our study are consistent with their finding that low vitamin D level predisposes patients to clinical relapse. However, their study did not control for UC disease severity or account for the effect of baseline inflammation in their multivariate analysis. Our study differs in that we restricted enrollment only to patients in clinical remission and adjusted for confounding effects of endoscopic and histologic inflammation in our analyses.

Previous studies also have shown that vitamin D levels are related inversely to UC disease activity. These studies of disease activity were based mainly on symptom scores and quality-of-life surveys.[18,19,31] Our study supports these previous findings that vitamin D is a marker of disease activity in UC and adds further to the literature by showing that vitamin D levels also reflect inflammatory activity at the endoscopic and histologic level beyond the clinical phenotype. Taken together, low serum vitamin D levels may be a useful biomarker for the detection of inflammation in UC patients in the absence of significant clinical symptoms.

Our finding that low vitamin D levels increases the risk of clinical relapse in UC patients in remission may be interpreted in several ways. One explanation is that the immunoprotective and anti-inflammatory properties of vitamin D diminishes with lower levels and this leads directly to subsequent inflammation and clinical relapse. Another explanation is that low vitamin D levels was an effect of increased disease activity and that the risk of clinical relapse was mediated through the effects of baseline inflammation. Low baseline vitamin D levels measured in our cohort were not caused by clinical disease activity because the patients enrolled in our study were in clinical remission. Although a subset of patients in our cohort had endoscopically and histologically active disease, our models adjusted for the effect of this baseline inflammation in our analysis. We also performed a subgroup analysis restricted only to patients in clinical and endoscopic remission, and after adjusting for histologic inflammation in our multivariate analysis, low vitamin D level still was associated with the risk of clinical relapse. Thus, our results show that low vitamin D level impacts risk of clinical relapse independent of subclinical inflammation.

Our study had several major strengths. First, our study was prospective and provided clarity of temporal sequence regarding our exposure of low vitamin D levels and outcome of clinical relapse. Second, our study was blinded: investigators measuring baseline serum vitamin D levels were blinded to subsequent relapse status to avoid selection bias at time of analysis and, likewise, our investigators assessing relapse during follow-up periods were blinded to baseline vitamin D levels. Third, our cohort was limited to UC patients in clinical remission, which allowed us to isolate the independent effects of vitamin D levels on risk of clinical relapse. Fourth, vitamin D status was based on direct vitamin D measurements from serum obtained at time of enrollment rather than from International Classification of Diseases, 9th revision, codes for vitamin D deficiency, random vitamin D values from the medical record, or from estimates of serum vitamin D calculated from diet, physical activity, and other predictors of vitamin D levels. Finally, our study focused solely on UC patients, a population that warrants greater attention regarding the clinical role of vitamin D. Generalizations regarding the effect of vitamin D on inflammatory bowel disease as a group may not be appropriate because of the genetic and immunologic differences in the pathogenesis of ulcerative colitis and Crohn's disease.

Our study had several limitations that merit attention. First, our sample size was small and may not be representative of the general UC population. Furthermore, the generalizability of our findings was limited only to UC patients in remission. Second, our study was based on a single measurement of serum vitamin D at time of enrollment, which may not reflect a patient's baseline levels and does not take into account the possibility of fluctuations over the course of 12 months. One large scale epidemiologic study by Jorde et al[32] involving long-term tracking of serum vitamin D levels acknowledged that although there are fluctuations in serum vitamin D

levels, they are unlikely to have substantial improvements over time. Their study supported the use of a single serum vitamin D measurement to predict future health outcomes. Third, our study was observational and thus was limited by the inability to account for potential unmeasured confounders. In particular, our study did not take into account baseline dietary vitamin D intake, body mass index, physical activity, parathyroid hormone levels, malabsorptive conditions, medications that affect vitamin D levels, or adherence to medical therapy. Finally, although our cohort enrolled patients with clinically quiescent UC, we did not restrict our enrollment to patients with endoscopic and histologic remission, which raises the possibility that subclinical inflammation rather than low vitamin D levels is contributing to risk of relapse. However, we adjusted for the confounding effects of baseline endoscopic and histologic inflammation in our subgroup analysis and multivariate models.

The results of our study have several clinical implications. First, although currently not part of regular surveillance and standard of care in ulcerative colitis patients, our findings support the need for routine measurement of serum vitamin D levels. Second, our study suggests a role of low vitamin D as a maker of endoscopic and histologic inflammation in the absence of significant clinical symptoms, which may in turn be used to risk-stratify patients. Third, our findings provide a rationale for vitamin D supplementation in maintenance therapy in patients with ulcerative colitis. Maintaining serum vitamin D levels greater than specified thresholds during periods of clinical remission may be protective against subsequent clinical relapse.

In conclusion, our study provides evidence that low vitamin D levels ($\leq$35 ng/mL) correlate with endoscopic and histologic inflammation and are associated with an increased risk of subsequent clinical relapse during periods of clinical remission. Vitamin D is an affordable, accessible, and relatively nontoxic supplement that may have protective effects in the maintenance of clinical remission in patients with ulcerative colitis. Clinical trials of vitamin D therapy to obtain vitamin D levels above this threshold should be considered to definitively establish its impact on ulcerative colitis outcomes.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2016.05.035.

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Conflicts of interest
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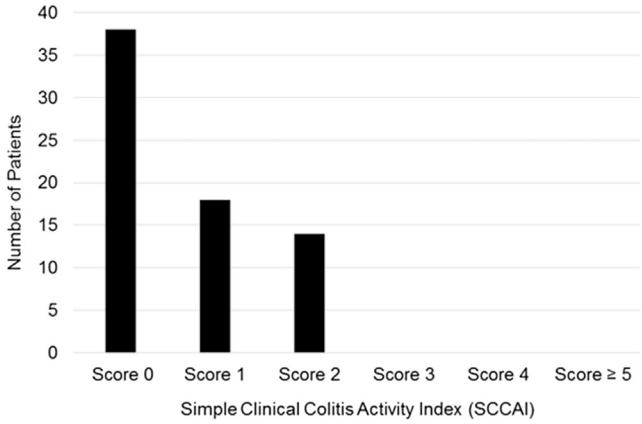

Supplementary Figure 1. Baseline SCCAI among prospective cohort (N = 70). All patients were in clinical remission with SCCAI of 2 or less at time of enrollment.

Supplementary Table 1. Effect of Serum Vitamin D Level Thresholds on Risk of Clinical Relapse Among Patients With Ulcerative Colitis in Clinical Remission

Serum vitamin D level, ≤*ng/mL*	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* value	OR	95% CI	*P* value
10	1.17	0.81–1.68	.401	1.22	0.88–1.70	.228
20	1.24	0.94–1.63	.134	1.18	0.92–1.52	.192
30	1.22	0.96–1.54	.096	1.08	0.86–1.35	.510
35	1.42	1.16–1.75	.001	1.27	1.03–1.57	.026
40	1.37	1.11–1.69	.003	1.21	0.98–1.49	.082
45	1.36	1.10–1.68	.005	1.24	1.01–1.52	.044
50	1.27	1.01–1.59	.040	1.16	0.93–1.44	.179
60	1.34	1.04–1.73	.022	1.21	0.96–1.54	.110
70	1.27	0.94–1.70	.116	1.23	0.94–1.60	.136