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Higher Serum Vitamin D Levels are Associated with Protective Serum Cytokine Profiles in Patients with Ulcerative Colitis

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Abstract

Background & Aims—Vitamin D has immune modulating effects on cytokines. Serum vitamin D levels are associated with the risk of relapse in patients with ulcerative colitis (UC), through unknown mechanisms. We tested the hypothesis that this beneficial role of vitamin D on UC is mediated through anti-inflammatory serum cytokine profiles.

Methods—Serum samples from a prospective cohort of seventy UC patients in clinical remission were collected and baseline histological and endoscopic scores were recorded at enrollment. Clinical relapse events were recorded over the 12-month follow-up period. Serum vitamin D and cytokines levels (IL-6, IL-8, IL-17A, TNF- α , IFN- γ , IL-4, IL-10) were quantified using ELISA. Linear regression was used to determine correlation between vitamin D and cytokine profiles. Logistic regression models were used to determine the association between serum cytokine profiles and baseline histologic mucosal healing and clinical relapse.

Results—Higher serum vitamin D levels positively correlated with higher ratios of IL-4+IL-10/IL-17A+TNF- α ($r=0.37$, $P<0.01$), and IL-4+IL-10/IL-6+TNF- α ($r=0.32$, $P<0.01$). In multivariate analysis, IL-4+IL-10/IL-17A+TNF- α ratios at baseline were associated with the presence of histologic mucosal healing (O.R. 1.29, 95% CI 1.02-1.62, $P=0.03$). A higher ratio of serum IL-4+IL-10 to IL-6+TNF- α was associated with a reduced risk of clinical relapse (O.R. 0.72, 95% CI 0.58-0.89, $P=0.003$), and longer time to relapse ($p=0.03$), over the 12 months follow-up. This ratio during remission had an AUC of 0.7 in predicting later clinical relapse.

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Conclusions—Vitamin D is associated with anti-inflammatory serum cytokine profiles. Anti-inflammatory cytokine patterns may mediate the protective effects of higher serum vitamin D levels in patients with ulcerative colitis.

Keywords

Vitamin D; Cytokines; Biomarker; Relapse; Mucosal Healing

Introduction

Cytokines are important mediators of inflammation. An imbalance between antiinflammatory and proinflammatory cytokines underlies the pathogenesis of various inflammatory disorders. [1,2,3]. Ulcerative colitis (UC) and Crohn's disease are inflammatory bowel diseases (IBD) that have been shown to have dysregulated systemic and intestinal cytokine responses [4,5,6]. Emerging therapeutic strategies are aimed at targeting cytokines or shifting the cytokine response to favor a more anti-inflammatory phenotype (e.g. Ustekinumab, Tofacitinib).

Vitamin D has been increasingly recognized for its immunomodulatory role and has been implicated in the pathogenesis of autoimmune disorders [7,8]. Vitamin D has been shown to have numerous and dynamic effects on the immune system, ranging from altering T cell responses [9,10] to influencing dendritic cell differentiation and protecting epithelial mucosal barriers against inflammation [11,12]. Vitamin D has also been shown to regulate cytokine pathways. In one *in vitro* study [13] involving peripheral blood mononuclear cells, vitamin D induced a dose-dependent, down regulation of proinflammatory cytokines IL-6, TNF- α , IL-17, and IFN- γ , while increasing the anti-inflammatory cytokine IL-10. Similar studies [14,15,16] demonstrated that vitamin D inhibited the production of proinflammatory cytokines (IL-6, TNF- α , IL-17, IL-21, IFN- γ) by macrophages and T cells. In another study [17], vitamin D promoted IL-10 production in human B cells.

Clinical studies have previously evaluated the effects of vitamin D on serum cytokine production in patients. In one randomized, controlled trial (RCT) involving healthy adult patients [18], supplemental vitamin D increased the serum concentration of IL-10 and IFN- γ in patients with initially low serum vitamin D. In two other RCTs [19,20], vitamin D supplementation resulted in increased serum levels of IL-10 in congestive heart failure patients, while TGF- β was increased in multiple sclerosis patients. Clinical studies evaluating the effect of vitamin D and cytokine profiles in IBD patients are lacking. One prospective study [21] in patients with Crohn's disease demonstrated that circulating levels of IL-10 were lower in patients with lower serum vitamin D levels, but did not link these to clinical outcomes. No current studies exist exploring vitamin D status and serum cytokine profiles in patients with ulcerative colitis.

The clinical impact of vitamin D status on IBD patients, especially in patients with ulcerative colitis has recently attracted significant attention. One recent prospective study [22] demonstrated that low vitamin D is associated with higher morbidity, disease severity, and healthcare utilization in IBD patients. In another study [23], serum vitamin D was found to be inversely associated with mucosal inflammation in UC patients. Our group recently

demonstrated that low serum vitamin D during remission increases the risk of UC clinical relapse independent of endoscopic or histologic grade [24]. Despite the increasing evidence of the benefits of vitamin D on ulcerative colitis, a mechanistic link between vitamin D and its immunoprotective effects in ulcerative colitis patients is not well understood.

Considering the effect that vitamin D has on cytokine production by circulating immune cells, and the imbalance in cytokine responses that underlie the pathogenesis of ulcerative colitis, we hypothesized that higher serum vitamin D is associated anti-inflammatory serum cytokine profiles and that this shift towards a more anti-inflammatory cytokine phenotype protects against clinical relapse. In this prospective study, we thus sought to determine the association between serum cytokine profiles and vitamin D levels during a period of clinical remission and their impact on presence of histologic mucosal healing at enrollment and subsequent risk of clinical relapse in ulcerative colitis patients.

Materials and Methods

Study Design and Patient Enrollment

We conducted a prospective study of patients with UC in clinical remission, defined by a Simple Clinical Colitis Activity Index (SCCAI) ≤ 2 [25,26], who were recruited after a surveillance colonoscopy from the Inflammatory Bowel Disease Center at Beth Israel Deaconess Medical Center (BIDMC), Harvard Medical School, Boston, MA from 2009-2012. The study was approved by the BIDMC institutional review board (IRB) under protocol # 2009P000314. A total of seventy patients who had baseline serum samples collected at the time of index colonoscopy were enrolled in the study. After the 12-month follow-up period was completed, blinded investigators (JG, SM) measured serum vitamin D and serum cytokine levels in these de-identified samples. Age, gender, ethnicity, smoking status, creatinine, duration of disease, extent of disease, relevant medications (current NSAIDs, current 5-ASA, current 6MP/AZA, current anti-TNF- 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, erythrocyte sedimentation rate, and C-reactive protein) were also obtained. (Table 1). Since this was an observational cohort study, we did not actively supplement patients with vitamin D. Furthermore, we did not assess for baseline dietary vitamin D intake or initiation of vitamin D supplementation anytime during the follow-up periods.

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 sub-score of the Mayo activity index based on the most inflamed segment of the colon. [27] Histological activity in all segments was classified using the Geboes score by a GI pathologist blinded to endoscopic scores. [28] For each patient, a total Geboes score was assigned to biopsies 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 (MES) ≥ 2 . Histologic inflammation was defined as a Geboes histologic score ≥ 3 . Histologic mucosal healing was defined by Geboes histologic score < 3 . "Clinical

relapse” during the follow-up periods (at 3, 6, and 12 months) was defined as a SCCAI score > 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 current regimen, addition of another medication, or change in class of medication due to symptom relapse. Of note, physicians assessing for clinical relapse were blinded to baseline endoscopic and histologic grade, serum vitamin D levels, and serum cytokine profiles.

Vitamin D Measurement

Serum vitamin D levels (25(OH)D) were measured by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Calbiotech, San Diego, CA) according to the manufacturer's instructions. From our previous study [24], we determined that a serum vitamin D cutoff of 35 ng/mL had the greatest association with clinical relapse. This serum cutoff was used in our univariate and multivariate logistic regression models for clinical relapse. To account for seasonal variations in vitamin D in New England, the year was dichotomized to a “low sunlight season” (September to February) and a “high sunlight season” (March to August). Sunlight season was included as a co-variate in our logistic regression analysis for risk of clinical relapse.

Multiplex Cytokine Measurement

A magnetic bead-based multiplex human cytokine assay (Milliplex, MAP Kit; EMD Millipore Corporation, St. Charles, MI) was used to simultaneously measure multiple cytokines (IL-6, IL-8, IL-17A, TNF- α , IFN- γ , IL-4, and IL-10) in patients' serum samples. Samples, standards (6-point dilution), and quality controls were mixed with antibody-linked magnetic beads on a 96-well plate and incubated overnight at 4°C with shaking. Plates were washed twice with Wash Buffer and well contents were removed by decanting with a hand-held magnet. Following a 1-hr incubation at room temperature (RT) with biotinylated detection antibodies, Streptavidin-Phycoerythrin was added and further incubated with a shaker for 30 minutes. Plates were washed as above, Drive Fluid was added to each well, and the plate was run on MAGPIX Multiplex Reader (Luminex Corporation, Austin, TX). The analysis was performed on xPONENT Software. All samples and standards were analyzed in duplicate, and plates were sealed and wrapped with aluminum foil at all times during incubation.

Serum Cytokine Profiles and Correlation with Serum Vitamin D

In this study, anti-inflammatory cytokines included IL-4 and IL-10 and proinflammatory cytokines included IL-6, IL-8, IL-17A, TNF- α , and IFN- γ . We constructed serum cytokine profiles which included individual serum cytokines levels, ratios of anti-inflammatory-to-proinflammatory cytokines, sums of anti-inflammatory cytokines, combinations of sums of proinflammatory cytokines, and ratios of sums of anti-inflammatory cytokines to sums of proinflammatory cytokines. (Table 2 and Supplementary Table 1) We plotted serum cytokine profiles as a function of serum vitamin D, performed linear regression analyses and calculated Pearson's r coefficient and p values using GraphPad Prism (version 7.01; GraphPad Software Inc., La Jolla, CA).

Serum Cytokine Profiles, Receiver Operator Characteristic (ROC) Curve, Univariate and Multivariate Analyses

We initially performed univariate linear regression analyses for each serum cytokine profile in order to define their effect on risk of baseline histologic mucosal healing and clinical relapse at 12 months. (Supplementary Table 1) Serum cytokine profiles with a trend towards association ($p < 0.300$) with histologic mucosal healing or clinical relapse were chosen for further analysis with a receiver operator characteristic (ROC) curve. We constructed ROC curves to determine the ability of each of these candidate cytokine profiles to identify patients with baseline histologic mucosal healing or predict the outcome of clinical relapse. For each ROC curve analysis, we calculated an area under the curve (AUC) and associated p values. (Table 3) The serum cytokine profiles with the highest AUC and lowest p values (greatest association with histologic mucosal healing or clinical relapse) were chosen for inclusion into our multivariate analyses. We determined serum cytokine ratio thresholds using data derived from the ROC curve analyses. Serum cytokine ratio thresholds with the greatest likelihood ratios to detect the presence of mucosal healing or predict clinical relapse were selected. Patients were dichotomized based on these thresholds which we used in our multivariate logistic regression models. Construction of ROC curves and calculations were performed using GraphPad Prism (version 7.01; GraphPad Software Inc., La Jolla, CA).

Statistical Analysis

The predictive value of clinical variables on presence of histologic mucosal healing or clinical relapse, odds ratio (OR) with its 95 % confidence interval (CI), and p - values were assessed using JMP Pro 12.0 (SAS Institute Inc., North Carolina). Dichotomous variables were analyzed for outcomes using χ^2 test or Fisher's exact test where appropriate, and continuous variables analyzed using t -test if normally distributed, or Wilcoxon test for non-normal data. Correction for multiple testing was included. All variables were initially analyzed in a univariate fashion to determine their association with baseline mucosal healing or clinical relapse. We included all co-variables in our multivariate logistic regression models for baseline histologic mucosal healing and risk of clinical relapse. Model building was based on forward stepwise logistic regression. We also performed a time-to-event analysis to detect a difference in time to clinical relapse among patients with a serum anti-inflammatory-to-proinflammatory cytokine ratio $L-4+IL-10/IL-6+TNF-\alpha > 13.03$ vs 13.03 (threshold based on ROC curve analysis). All figures were generated using GraphPad Prism (version 7.01; GraphPad Software Inc., La Jolla, CA).

Results

Baseline Patient Characteristics and Clinical Relapse at 12 Months

Table 1 details the baseline phenotype of the enrolled cohort. The mean serum Vitamin D level in the cohort was 44 ng/mL (± 29). Only 8 patients (11.4%) had steroid use in the past year. Vitamin D supplementation use was reported by 42 patients (60%). Among the seventy patients enrolled, endoscopic inflammation was present in 9 patients (13%), while histologic inflammation was present in 32 patients (46%) at baseline. At the end of the 12-month follow-up period, 20 patients relapsed while 50 patients remained in clinical remission. All patients who later relapsed had a SCCAI > 2 at follow-up. Clinical relapse outcomes in our

cohort included: SCCAI >2 alone (N= 5), SCCAI >2 with resulting medication intensification (N=13), and SCCAI >2 with resulting medication intensification and hospitalization (N= 2).

Baseline Serum Cytokine Profiles

Table 2 summarizes mean baseline serum cytokine levels, among ulcerative colitis patients based on relapse status at 12 months and presence of baseline mucosal healing or inflammation at enrollment. Mean TNF- α levels were higher among patients who remained in remission vs relapsers: 10.85 vs 7.31, $p=0.035$. The mean IL-4+IL-10/IL-6+TNF- α ratio was higher among patients in remission vs relapse at 12 months: 23.77 vs 12.54 ($p=0.048$). (Figure 1)

Serum Vitamin D and Serum Cytokine Profile Correlations

In our linear regression analyses, serum vitamin D correlated with serum IL-10 levels ($r=0.3335$, $p<0.01$) and serum IL-4+IL-10 ($r=0.2611$, $p=0.03$). Serum vitamin D did not correlate with IL-4 or any of the proinflammatory cytokines (IL-6, IL-8, IL-17A, TNF- α). (Supplementary Figure 1). Higher serum vitamin D levels correlated with increased serum anti-inflammatory-to-proinflammatory cytokine ratios IL-4+IL-10/IL-17A+TNF- α ($r=0.3559$, $P<0.01$) and IL-4+IL-10/IL-6+TNF- α ($r=0.3249$, $P<0.01$). (Figure 2).

Univariate Analysis of Baseline Serum Cytokine Profiles for Presence of Baseline Mucosal Healing and Risk of Clinical Relapse

Supplementary Table 1 summarizes the univariate analysis of serum cytokine profiles for the presence of baseline histologic mucosal healing and risk of clinical relapse. No individual serum cytokine (per 1 pg/mL) or serum cytokine ratio (continuous data, not dichotomized based on threshold) significantly correlated with either presence of histologic mucosal healing or risk of clinical relapse at 12 months.

Serum cytokine profiles with a trend towards association (defined as $p<0.300$) were further analyzed in ROC curve analyses to determine thresholds of serum cytokine profiles that were significantly associated with either mucosal healing or clinical relapse (Table 3). Among the serum cytokine profiles selected, serum cytokine ratio IL-4+IL-10/IL-17A+TNF- α had the greatest ability to identify UC patients with baseline histologic mucosal healing with an area under the curve (AUC) of 0.641, $p=0.044$. A serum cytokine ratio IL-4+IL-10/IL-17A+TNF- α threshold of 0.1522 had the greatest sensitivity (52.6%, 95% CI 35.8%-69.0%) and specificity (75.0%, 95% CI 56.6%-88.5%) with a likelihood ratio of 2.015 (Table 3). We thus used a threshold of 0.1522 in our logistic regression analyses for presence of histologic mucosal healing. Among the serum cytokine profiles selected for ROC curve analyses for outcome of clinical relapse, serum cytokine ratio IL-4+IL-10/IL-6+TNF- α had the greatest ability to predict risk of clinical relapse with an AUC of 0.700, $p=0.012$ (Figure 3A). A serum cytokine ratio IL-4+IL-10/IL-6+TNF- α threshold of 13.03 had the greatest sensitivity (55%, 31.5%-76.9%) and specificity (82%, 68.6%-91.4%) with a likelihood ratio of 3.06 for predicting clinical relapse at 12 months. We used a threshold of 13.03 in our logistic regression models for the outcome of clinical relapse.

Multivariate Analysis of Presence of Baseline Histologic Mucosal Healing

Table 4 summarizes the clinical factors associated with presence of histologic mucosal healing in our multivariate model. In multivariate analysis, factors associated with increased presence of histologic mucosal healing included current smoking (OR 2.88, 95% CI 1.33-6.21, $p=0.008$) and serum cytokine ratio IL-4+IL10/IL-17A+TNF- α > 0.1522 (OR 1.29, 95% CI 1.02-1.62, $p=0.032$). Factors associated with decreased risk of mucosal healing included a serum vitamin D $\leq$ 35 ng/mL (OR 0.76, 95% CI 0.59-0.99, $p=0.043$) and steroid use in the past year (OR 0.65, 95% CI 0.42-0.99, $p=0.046$). UC patients with IL-4+IL10/IL-17A+TNF- α > 0.1522 had greater baseline histologic mucosal healing compared to patients with a ratio $\leq$ 0.1522: 71.4% (20/28 patients) vs 42.9% (18/42 patients), $p=0.017$.

Multivariate Analysis of Risk of Clinical Relapse

Table 5 summarizes the clinical factors associated with risk of clinical relapse in our multivariate model. In multivariate analysis, only baseline histologic inflammation was associated with increased (OR 1.37, 95% CI 1.06-1.78, $p=0.019$) risk of clinical relapse, while a baseline serum cytokine ratio IL-4+IL-10/IL-6+TNF- α > 13.03 was associated with decreased (OR 0.72, 95% CI 0.58-0.89) risk of clinical relapse. Serum vitamin D was no longer associated with risk of clinical relapse when we included serum cytokine ratio IL-4+IL-10/IL-6+TNF- α in our multivariate model. UC patients with IL-4+IL-10/IL-6+TNF- α > 13.03 had decreased rate of clinical relapse compared to UC patients with a ratio $\leq$ 13.03: 17.1% (6/35 patients) vs 40.0% (14/35 patients), $p=0.035$. Furthermore, in our time-to-event analysis, UC patients with a serum cytokine ratio IL-4+IL-10/IL-6+TNF- α $\leq$ 13.03 had an increased time to clinical relapse ($p=0.03$ by Log-rank Mantel-Cox test) compared to patients with a ratio > 13.03 (Figure 3B).

Discussion

In this prospective cohort study of patients with ulcerative colitis, we demonstrate for the first time that higher serum vitamin D levels positively correlate with serum anti-inflammatory cytokine ratios, and clinical outcomes. Higher anti-inflammatory cytokine ratios during a period of remission are associated with increased presence of histologic mucosal healing, and decreased risk of longitudinal clinical relapse in multivariate analysis.

Although the effect of vitamin D on serum cytokine release from peripheral immune cells has been studied in other disease models, the role of vitamin D on cytokine profiles in ulcerative colitis has not been critically examined. To our knowledge, this is the first study establishing an association between serum vitamin D levels and serum cytokine profiles in UC patients. One previous cross-sectional observational study [23] demonstrated that low serum vitamin D levels are associated with decreased mucosal mRNA expression of TNF- α and IL-8 in UC patients. However, the study only measured mRNA of two cytokines and did not link cytokine mRNA levels to future UC clinical outcomes.

The results of our study indicate that higher serum vitamin D correlated with increasing levels of serum IL-10, which is consistent with experimental *in vitro* models [13,17] as well human studies with vitamin D supplementation. [18,19]. Furthermore, our results parallel

one study [21] involving Crohn's disease patients which demonstrated an association between serum vitamin D and IL-10 levels. Serum and mucosal cytokine profiles in ulcerative colitis have been previously shown to correlate with disease activity and clinical outcomes. One previous study [29] involving patients with moderate to severe UC demonstrated that serum IL-8 levels correlated with endoscopic and histologic activity. Another study [30] demonstrated that UC patients with therapy failure with anti-TNF treatment have a more severe proinflammatory cytokine profile (IL-1 β , IL-6, and IL-17A) prior to therapy. Our study, which enrolled only UC patients in clinical remission, did not show an association between serum IL-8 levels and histologic activity or risk of clinical relapse. Previous studies with UC patients in remission [31,32] have shown no association between serum cytokine levels and clinical relapse. Our findings are consistent with these previous studies that no individual cytokine level was associated with clinical relapse. However, these previous studies did not measure anti-inflammatory cytokines or examine the net effect of anti-inflammatory and proinflammatory cytokines.

Our finding that higher serum vitamin D is associated with a more anti-inflammatory cytokine phenotype could be interpreted in several ways. One explanation is that higher serum vitamin D increases the production of anti-inflammatory cytokines relative to proinflammatory cytokines. Although our study was observational and cannot directly establish causality, previous interventional *in vitro* and clinical studies [13,17,18,19] provide evidence that vitamin D supplementation increases anti-inflammatory cytokines. Another explanation is that lower serum vitamin D level is a consequence, rather than a cause, of the chronic elevation of inflammatory cytokines. In our cohort, the temporal relationship between serum vitamin D and cytokine profiles is unclear. Clinical trials evaluating the effect of vitamin D supplementation on serum cytokine profiles and clinical relapse among ulcerative colitis patients are thus warranted.

Another major finding in our study was that a more anti-inflammatory cytokine phenotype during remission protects against future clinical relapse. Serum anti-inflammatory to proinflammatory cytokine ratios may represent a novel predictor of ulcerative colitis clinical relapse beyond the endoscopic and histologic phenotype. Indeed, in one study by Zenlea *et al* [33], even ulcerative colitis patients in clinical, endoscopic, and histologic remission had a clinical relapse rate of about 7%. Based on our findings, we speculate that preceding serum cytokine imbalances towards a proinflammatory phenotype may be a prelude for future histologic inflammation and clinical relapse.

Our study has several major strengths. First, our study was prospective and clearly established a baseline serum anti-inflammatory to proinflammatory cytokine ratio exposure preceding the outcome of clinical relapse. Second, our study was physician- and investigator-blinded: physicians assessing for relapse status at our follow-up periods were blinded to baseline serum vitamin D and cytokine profiles. Likewise, investigators measuring serum vitamin D and cytokine levels were also blinded to clinical outcomes. The de-identified serum samples were not combined with their corresponding clinical outcomes data until after the study period had ended. Third, our study quantified the ratios of anti-inflammatory to proinflammatory cytokines rather than focusing on individual cytokine

levels, which allowed us to explore the net effect of the cytokine imbalance which has been implicated in the pathogenesis of IBD.

Our study has several limitations that merit attention. First, our sample size was small and only included UC patients in clinical remission. This limits the generalizability of our findings to only ulcerative colitis patients in remission. Second, our study was based on a single measurement of serum vitamin D and cytokine levels at time of enrollment. However, previous epidemiological studies have shown that serum vitamin D [34] and cytokine measurements [35,36,37] from a single time point have acceptable temporal stability, reliability, and reproducibility for prospective studies. We also did not measure 1,25(OH)₂D₃ levels, which may provide additional insights into whether there are differences in the physiologically active form in patients with UC. Third, our study is observational and limited by the inability to account for potential unmeasured confounders. Although our study adjusted for baseline vitamin D supplementation in our UC patients, we did not take into account baseline dietary vitamin D intake, BMI, physical activity, medications that may affect vitamin D levels, adherence to medical therapy, or co-morbid inflammatory conditions other than IBD. Notably, current vitamin D supplementation did not independently associate with the clinical outcomes, addressing the potential of supplement use to confound the results.

Our study has several clinical implications. First, our findings propose a potential mechanistic link between serum vitamin D and its immunoprotective effects on ulcerative colitis clinical outcomes. If higher serum vitamin D levels encourage a more anti-inflammatory serum cytokine profile in patients with UC, this supports the importance of maintaining adequate levels of vitamin D to maintain clinical remission in patients with UC. Second, our study provides a rationale for further exploring serum cytokine profiles, rather than individual cytokine levels, as biomarkers of histological remission. Serum anti-inflammatory to proinflammatory cytokine ratios may also be used to identify UC patients who are at greater risk of clinical relapse.

In summary, our study demonstrates that in a prospective cohort of ulcerative colitis patients in clinical remission, higher serum vitamin D correlates with greater serum anti-inflammatory to proinflammatory cytokine ratios, and these anti-inflammatory cytokine phenotypes are associated with increased presence of histologic mucosal healing and decreased risk of clinical relapse. Our data provide further evidence for the anti-inflammatory effect of vitamin D on ulcerative colitis and suggest that the protective effects of vitamin D on risk of relapse may be partially cytokine-mediated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Feghali CA, Wright TM. Cytokines in acute and chronic inflammation. *Front Biosci.* 1997; 2(1):d12–d26. [PubMed: 9159205]
2. Dinarello CA. Role of pro-and anti-inflammatory cytokines during inflammation: experimental and clinical findings. *Journal of biological regulators and homeostatic agents.* 1996; 11(3):91–103.
3. Ward, Peter A. Cytokines, inflammation, and autoimmune diseases. *Hospital Practice.* 1995; 30.5:35–41.
4. Mizoguchi A, Mizoguchi E, Chiba C, Spiekermann GM, Tonegawa S, Nagler-Anderson C, Bhan AK. Cytokine imbalance and autoantibody production in T cell receptor-alpha mutant mice with inflammatory bowel disease. *The Journal of experimental medicine.* 1996; 183(3):847–856. [PubMed: 8642289]
5. Niessner M, Volk BA. Altered Th1/Th2 cytokine profiles in the intestinal mucosa of patients with inflammatory bowel disease as assessed by quantitative reversed transcribed polymerase chain reaction (RT-PCR). *Clinical & Experimental Immunology.* 1995; 101.3:428–435. [PubMed: 7664489]
6. Rogler, Gerhard, Andus, Tilo. Cytokines in inflammatory bowel disease. *World journal of surgery.* 1998; 22.4:382–389. [PubMed: 9523521]
7. Cantorna, Margherita T., Mahon, Brett D. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. *Experimental biology and medicine.* 2004; 229.11:1136–1142. [PubMed: 15564440]
8. Ooi, Jot Hui, Jing, Chen, Cantorna, Margherita T. Vitamin D regulation of immune function in the gut: why do T cells have vitamin D receptors? *Molecular aspects of medicine.* 2012; 33.1:77–82. [PubMed: 22079836]
9. Sloka, Scott, et al. Predominance of Th2 polarization by vitamin D through a STAT6-dependent mechanism. *J Neuroinflammation.* 2011; 8.1:56–66. [PubMed: 21605467]
10. Cantorna, Margherita T., et al. Vitamin D and 1, 25 (OH) 2D regulation of T cells. *Nutrients.* 2015; 7.4:3011–3021. [PubMed: 25912039]
11. Piemonti, Lorenzo, et al. Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells. *The Journal of Immunology.* 2000; 164.9:4443–4451. [PubMed: 10779743]
12. Liu, Weicheng, et al. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. *The Journal of clinical investigation.* 2013; 123.9:3983–3996. [PubMed: 23945234]
13. Khoo AL, Chai LY, Koenen HJ, Kullberg BJ, Joosten I, van der Ven AJ, Netea MG. 1, 25-dihydroxyvitamin D3 modulates cytokine production induced by *Candida albicans*: impact of seasonal variation of immune responses. *Journal of Infectious Diseases.* 2011; 203(1):122–130. [PubMed: 21148505]
14. Zhang, Yong, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *The Journal of Immunology.* 2012; 188.5:2127–2135. [PubMed: 22301548]
15. Jeffery LE, Burke F, Mura M, Zheng Y, Qureshi OS, Hewison M, Sansom DM, et al. 1, 25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *The Journal of Immunology.* 2009; 183(9):5458–5467. [PubMed: 19843932]
16. Chang SH, Chung Y, Dong C. Vitamin D suppresses Th17 cytokine production by inducing C/EBP homologous protein (CHOP) expression. *Journal of Biological Chemistry.* 2010; 285(50):38751–38755. [PubMed: 20974859]
17. Heine G, Niesner U, Chang HD, Steinmeyer A, Zügel U, Zuberbier T, Worm M, et al. 1, 25-dihydroxyvitamin D3 promotes IL-10 production in human B cells. *European journal of immunology.* 2008; 38(8):2210–2218. [PubMed: 18651709]
18. Barker, Tyler, et al. Supplemental vitamin D increases serum cytokines in those with initially low 25-hydroxyvitamin D: a randomized, double blind, placebo-controlled study. *Cytokine.* 2015; 71.2:132–138. [PubMed: 25461390]

19. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *The American journal of clinical nutrition*. 2006; 83(4):754–759. [PubMed: 16600924]
20. Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. *Journal of neuroimmunology*. 2003; 134(1):128–132. [PubMed: 12507780]
21. Kelly P, Suibhne TN, O'Morain C, O'Sullivan M. Vitamin D status and cytokine levels in patients with Crohn's disease. *International Journal for Vitamin and Nutrition Research*. 2011; 81(4):205. [PubMed: 22237768]
22. Kabbani TA, Koutroubakis IE, Schoen RE, Ramos-Rivers C, Shah N, Swoger J, Baidoo L, et al. Association of vitamin D level with clinical status in inflammatory bowel disease: a 5-year longitudinal study. *The American journal of gastroenterology*. 2016
23. Meckel K, Li YC, Lim J, Kocherginsky M, Weber C, Almoghrabi A, Cohen RD, et al. Serum 25-hydroxyvitamin D concentration is inversely associated with mucosal inflammation in patients with ulcerative colitis. *The American journal of clinical nutrition*. 2016 ajcn123786.
24. Gubatan J, Mitsuhashi S, Zenlea T, Rosenberg L, Robson S, Moss AC. Low Serum Vitamin D During Remission Increases Risk of Clinical Relapse in Patients with Ulcerative Colitis. *Clinical Gastroenterology and Hepatology*. 2016
25. Walmsley RS, Ayres RCS, Pounder RE, Allan RN. A simple clinical colitis activity index. *Gut*. 1998; 43(1):29–32. [PubMed: 9771402]
26. Higgins PDR, Schwartz M, Mapili J, Krokos I, Leung J, Zimmermann EM. Patient defined dichotomous end points for remission and clinical improvement in ulcerative colitis. *Gut*. 2005; 54(6):782–788. [PubMed: 15888785]
27. Turner, Dan, et al. A systematic prospective comparison of noninvasive disease activity indices in ulcerative colitis. *Clinical Gastroenterology and Hepatology*. 2009; 7.10:1081–1088. [PubMed: 19577010]
28. Geboes, Karel, et al. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. *Gut*. 2000; 47.3:404–409. [PubMed: 10940279]
29. Rodríguez-Perlvárez ML, García-Sánchez V, Villar-Pastor CM, González R, Iglesias-Flores E, Muntane J, Gómez-Camacho F. Role of serum cytokine profile in ulcerative colitis assessment. *Inflammatory bowel diseases*. 2012; 18(10):1864–1871. [PubMed: 22238172]
30. Dahlén R, Magnusson MK, Bajor A, Lasso A, Ung KA, Strid H, Öhman L. Global mucosal and serum cytokine profile in patients with ulcerative colitis undergoing anti-TNF therapy. *Scandinavian journal of gastroenterology*. 2015; 50(9):1118–1126. [PubMed: 25877762]
31. García-Sánchez V, González R, Iglesias-Flores E, Gisbert JP, Ángel-Rey JM, Soto-Escribano P, ... & Muntané J. Can systemic cytokines predict relapse of inflammatory bowel disease? *Hepato-gastroenterology*. 2009; 57(99-100):524–530.
32. Yamamoto T, Umegae S, Kitagawa T, Matsumoto K. Systemic and local cytokine production in quiescent ulcerative colitis and its relationship to future relapse: a prospective pilot study. *Inflammatory bowel diseases*. 2005; 11(6):589–596. [PubMed: 15905707]
33. Zenlea T, Yee EU, Rosenberg L, Boyle M, Nanda KS, Wolf JL, Moss AC, et al. Histology grade is independently associated with relapse risk in patients with ulcerative colitis in clinical remission: a prospective study. *The American journal of gastroenterology*. 2016
34. Jorde R, Sneve M, Hutchinson M, Emaus N, Figenschau Y, Grimnes G. Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. *American journal of epidemiology*. 2010; 171(8):903–908. [PubMed: 20219763]
35. Hosnijeh FS, Krop EJ, Portengen L, Rabkin CS, Linseisen J, Vineis P, Vermeulen R. Stability and reproducibility of simultaneously detected plasma and serum cytokine levels in asymptomatic subjects. *Biomarkers*. 2010; 15(2):140–148. [PubMed: 19848603]
36. Hofmann JN, Yu K, Bagni RK, Lan Q, Rothman N, Purdue MP. Intra-individual variability over time in serum cytokine levels among participants in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cytokine*. 2011; 56(2):145–148. [PubMed: 21764327]

37. Epstein MM, Breen EC, Magpantay L, Detels R, Lepone L, Penugonda S, Birmann BM, et al. Temporal stability of serum concentrations of cytokines and soluble receptors measured across two years in low-risk HIV-seronegative men. *Cancer Epidemiology Biomarkers & Prevention*. 2013; 22(11):2009–2015.

Abbreviations

IBD	inflammatory bowel disease
UC	ulcerative colitis, Simple Colitis Clinical Activity Index, SCCAI
OR	Odds Ratio
ROC	receiver operating characteristic
AUC	area under the curve
NSAIDs	nonsteroidal anti-inflammatory drugs, 5ASA, 5-aminosalicylic acid
6MP/AZA	6-mercaptopurine/azathioprine
anti-TNF-α	anti-tumor necrosis factor- α , IL, interleukin
IFN-γ	interferon-gamma
RCT	randomized controlled trial

Highlights

- Positive correlation between serum vitamin D (25(OH)D) levels and anti-inflammatory serum cytokine profiles
- Serum cytokine profile IL-4+IL-10/IL-17A+ TNF- α is associated with presence of histologic mucosal healing among ulcerative colitis patients in clinical remission
- Baseline serum cytokine profile IL-4+IL-10/IL-6+TNF- α during periods of clinical remission predicts longitudinal risk of relapse in ulcerative colitis patients

Higher Baseline Serum Cytokine IL-4+IL-10/IL-6+TNF- α Ratios Favor Maintenance of Clinical Remission at 12 Months

Figure 1.

The mean IL-4+IL-10/IL-6+TNF- α ratio was higher among patients in remission vs relapse at 12 months: 23.77 vs 12.54 ($P < 0.05$)

Figure 2.

Correlation between serum vitamin D levels and cytokine profiles. (A) Serum vitamin D levels correlated with serum levels of anti-inflammatory cytokine IL-10 ($r = 0.3335$, $p < 0.01$). (B) Serum vitamin D levels correlated with serum IL-4+IL-10/IL-17A+TNF- α ratios ($r = 0.3659$, $p < 0.01$) (C) Serum vitamin D levels correlated with serum IL-4+IL-10/IL-6+TNF- α ratios ($r = 0.3249$, $p < 0.01$).

Figure 3.

(A) Receiver Operator Characteristic (ROC) Curve for cytokine IL-4+IL-10/IL-6+TNF- α ratio on the outcome of clinical relapse. Area under curve (AUC) was 0.700, $p=0.012$. A ratio of > 13.03 had a likelihood ratio of 3.06, sensitivity of 55% (31.5%-76.9%), and specificity of 82% (68.6%-91.4%) for predicting clinical relapse at 12 months. (B) Time to Event Analysis of Effect of Cytokine Ratio IL-4+IL-10/IL-6+TNF- α on Clinical Relapse at 12 Months. Ulcerative colitis patients with a serum cytokine ratio IL-4+IL-10/IL-6+TNF- α > 13.03 at enrollment had a decreased time to clinical relapse ($p=0.03$ by Log-rank Mantel-Cox).

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

Clinical Characteristics	Number of Subjects (Percentage)
<i>Demographics</i>	
Average Age (years)	48.6 ($\pm$ 15.2)
Female Gender	45 (64.3)
Caucasian Ethnicity	65 (92.9)
Smoking (Current) ^a	2 (2.9)
Average Creatinine (mg/dL)	0.86 ($\pm$ 0.19)
Season of Enrollment (Low Sunlight) ^b	15 (21.4)
<i>Ulcerative Colitis Characteristics</i>	
Disease Duration (average years)	12.0 ($\pm$ 13.6)
Left-Sided Colitis	27 (38.6)
Extensive Colitis	37 (52.9)
Duration of Remission $\geq$ 6 months	57 (81.4)
<i>Medications</i>	
Current NSAIDs	7 (10)
Current 5ASA	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)
<i>Baseline Lab Values (Mean $\pm$ SD)</i>	
White Blood Cell (K/uL)	6.7 ($\pm$ 2.3)
Hematocrit (%)	41.0 ($\pm$ 5.0)
Erythrocyte Sedimentation Rate (mm/hr)	9.6 ($\pm$ 9.3)
C-Reactive Protein (mg/L)	3.2 ($\pm$ 4.7)
Serum Vitamin D (ng/mL) ^d	44.0 ($\pm$ 29)
<i>Baseline Inflammation</i>	
Endoscopic Inflammation ^e	9 (12.9)
Histologic Inflammation ^f	32 (45.7)

^aThere were only two patients who were current smokers at start of study, no patients were former smokers

^bLow sunlight season in Massachusetts (September to February), high sunlight season (March to August)

^cIncludes patients who were on baseline Vitamin D supplements (including Multi-Vitamin) at time of enrollment in study

^dBaseline serum Vitamin D level at time of enrollment in study

^eEndoscopic Inflammation defined as Mayo Endoscopic Score $\geq$ 2

^fHistologic Inflammation defined as Geboes histologic score of $\geq$ 3

Table 2
Summary of Serum Cytokines Values According to Clinical Relapse at 12 Months and Mucosal Healing

Serum Cytokines (pg/mL for levels, no units for ratios)	CLINICAL RELAPSE AT 12 MONTHS			HISTOLOGIC MUCOSAL HEALING		
	Remission (mean $\pm$ SD)	Relapse (mean $\pm$ SD)	p Value	Mucosal Healing (mean $\pm$ SD)	Mucosal Inflammation (mean $\pm$ SD)	p Value
IL-6	2.33 ($\pm$ 4.57)	0.91 ($\pm$ 3.06)	0.137	2.26($\pm$ 5.01)	1.53 ($\pm$ 3.05)	0.456
IL-8	33.58($\pm$ 52.67)	29.74($\pm$ 56.32)	0.778	33.60($\pm$ 46.45)	31.15($\pm$ 58.02)	0.848
IL-17A	4.64($\pm$ 8.98)	4.62($\pm$ 10.17)	0.994	3.39($\pm$ 7.31)	6.11 ($\pm$ 11.09)	0.241
TNF- α	10.85($\pm$ 7.60)	7.31 ($\pm$ 5.55)	0.035	10.26($\pm$ 8.22)	9.34($\pm$ 5.84)	0.591
IFN- γ	18.23($\pm$ 36.14)	15.40($\pm$ 48.45)	0.806	13.61 ($\pm$ 27.20)	21.96($\pm$ 49.12)	0.396
IL-4	4.49($\pm$ 13.05)	1.11 ($\pm$ 3.41)	0.095	3.37($\pm$ 7.90)	3.70($\pm$ 14.39)	0.908
IL-10	6.10($\pm$ 13.84)	3.19($\pm$ 8.41)	0.289	6.42($\pm$ 14.83)	3.90($\pm$ 9.17)	0.389
IL-4+IL-10	10.59($\pm$ 20.75)	4.30($\pm$ 11.41)	0.111	9.79($\pm$ 19.67)	7.61 ($\pm$ 17.73)	0.538
IL4+IL-10/IL-6+TNF- α	23.77($\pm$ 28.78)	12.53($\pm$ 16.96)	0.048	22.31 ($\pm$ 29.60)	18.48($\pm$ 22.13)	0.136
IL-4+IL-10/IL-17A+TNF- α	0.89($\pm$ 1.84)	0.46($\pm$ 0.99)	0.212	0.98($\pm$ 2.02)	0.43($\pm$ 0.95)	0.626

Table 3
Receiver Operating Characteristic (ROC) Curve Analysis for the Presence of Histologic Mucosal Healing and Outcome of Clinical Relapse in 12 Months

Serum Cytokine Profile	Area Under Curve (AUC)	p Value
<i>HISTOLOGIC MUCOSAL HEALING^a</i>		
IL-17A	0.54	0.532
IL-10/IL-17A	0.51	0.878
IL-4+IL10/IL-17A	0.54	0.540
IL-4+IL-10/IL-6+TNF- α	0.54	0.604
IL-4+IL10/IL-17A+TNF- α	0.64	0.044
<i>CLINICAL RELAPSE IN 12 MONTHS^b</i>		
TNF- α	0.63	0.090
IL-4	0.58	0.292
IL-10	0.60	0.210
IL-4/TNF- α	0.58	0.298
IL-4+IL-10/IL-17A+TNF- α	0.65	0.054
IL-4+IL-10	0.65	0.048
IL-4+IL-10/IL-8	0.66	0.038
IL-4+IL-10/IL-8+TNF- α	0.67	0.029
IL-4+IL-10/IL-6+TNF- α	0.70	0.012

^aHistologic Mucosal Healing defined by Geboes Histologic Grade of < 3

^bClinical Relapse defined by SCCAI >2, medication intensification, or UC-related hospitalization anytime during 12-month followup

Table 4
Analysis of Factors Associated with Presence of Histologic Mucosal Healing^a

Clinical Variables	UNIVARIATE			MULTIVARIATE				
	Odds Ratio	95% CI	p Value	Odds Ratio	95% CI	p Value		
Age (per year)	1.01	1.00	1.02	0.072	1.00	1.01	0.352	
Female Gender	1.00	0.78	1.29	0.989	0.99	0.77	1.26	0.921
Caucasian Ethnicity	0.77	0.59	0.99	0.044	0.96	0.75	1.22	0.709
Smoking (Current)	1.60	0.78	3.27	0.193	2.88	1.33	6.21	0.008
Season (Low Sunlight) ^b	0.90	0.71	2.46	0.384	1.03	0.83	1.27	0.784
Vitamin D level ≥ 35 ng/mL	0.63	0.51	0.79	<.0001	0.76	0.59	0.99	0.043
IL-4+IL-10/IL-17A+ TNF- α > 0.1522	1.33	1.05	1.69	0.019	1.29	1.02	1.62	0.032
Current NSAIDs	0.88	0.59	1.31	0.529	0.96	0.65	1.40	0.815
Current 5ASA	0.81	0.61	1.06	0.125	0.85	0.66	1.10	0.220
Current 6MP/AZA	0.87	0.64	1.17	0.344	0.90	0.68	1.19	0.461
Current anti-TNF- α	1.25	0.74	2.09	0.399	1.32	0.82	2.13	0.246
Current Vitamin D Supplementation	1.17	0.92	1.50	0.196	1.04	0.81	1.32	0.771
Steroids in past year	0.72	0.50	1.04	0.079	0.65	0.42	0.99	0.046
Endoscopic Inflammation ^c	0.54	0.39	0.75	<.001	0.73	0.51	1.03	0.073

^aHistologic Mucosal Healing defined by Geboes Histologic Grade ≤ 3

^bLow sunlight season in Massachusetts (September to February), high sunlight season (March to August)

^cEndoscopic Inflammation defined by Mayo Endoscopic Score ≥ 2

Table 5
Analysis of Factors Associated with Clinical Relapse in 12 Months

Clinical Variables	UNIVARIATE			MULTIVARIATE				
	Odds Ratio	95% CI	p Value	Odds Ratio	95% CI	p Value		
Age (per year)	0.99	0.99	1.00	0.146	1.26	0.69	2.29	0.448
Female Gender	1.06	0.84	1.34	0.601	1.00	0.99	1.00	0.478
Caucasian Ethnicity	1.15	0.90	1.46	0.257	1.17	0.92	1.49	0.193
Smoking (Current)	1.24	0.64	2.40	0.513	0.96	0.75	1.22	0.726
Season (Low Sunlight) ^a	1.20	0.97	3.32	0.097	1.08	0.87	1.33	0.477
Vitamin D level 35 ng/mL	1.26	1.16	1.75	0.001	1.12	0.86	1.46	0.405
IL-4+IL-10/IL-6+ TNF-α > 13.03	0.80	0.64	0.98	0.035	0.72	0.58	0.89	0.003
Current NSAIDs	1.00	0.69	1.44	0.980	0.91	0.63	1.33	0.628
Current 5ASA	1.26	0.98	1.61	0.073	1.09	0.84	1.41	0.512
Current 6MP/AZA	1.12	0.85	1.49	0.411	0.99	0.75	1.31	0.934
Current anti-TNF-α	0.96	0.60	1.54	0.859	1.00	0.63	1.58	0.988
Current Vitamin D Supplementation	0.87	0.70	1.09	0.220	0.97	0.76	1.23	0.786
Steroids in past year	1.46	1.05	2.04	0.026	1.23	0.80	1.89	0.341
Endoscopic Inflammation ^b	1.35	0.98	1.87	0.066	1.02	0.71	1.45	0.920
Histologic Inflammation ^c	1.52	1.22	1.89	<0.001	1.37	1.06	1.78	0.019

^aLow sunlight season in Massachusetts (September to February), high sunlight season (March to August)

^bEndoscopic Inflammation defined by Mayo Endoscopic Score

^cHistologic Inflammation defined by Geboes Histologic Grade of