Higher plasma vitamin D is associated with reduced risk of *Clostridium difficile* infection in patients with inflammatory bowel diseases

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SUMMARY

Background

Patients with inflammatory bowel diseases (IBD) have an increased risk of *clos-tridium difficile* infection (CDI). Cathelicidins are anti-microbial peptides that attenuate colitis and inhibit the effect of clostridial toxins. Plasma calcifediol [25 (OH)D] stimulates production of cathelicidins.

Aim

To examine the association between plasma 25(OH)D and CDI in patients with IBD.

Methods

From a multi-institutional IBD cohort, we identified patients with at least one measured plasma 25(OH)D. Our primary outcome was development of CDI. Multivariate logistic regression models adjusting for potential confounders were used to identify independent effect of plasma 25(OH)D on risk of CDI.

Results

We studied 3188 IBD patients of whom 35 patients developed CDI. Patients with CDI-IBD were older and had greater co-morbidity. The mean plasma 25(OH)D level was significantly lower in patients who developed CDI (20.4 ng/mL) compared to non-CDI-IBD patients (27.1 ng/mL) (P = 0.002). On multivariate analysis, each 1 ng/mL increase in plasma 25(OH)D was associated with a 4% reduction in risk of CDI (OR 0.96, 95% CI 0.93–0.99, P = 0.046). Compared to individuals with vitamin D >20 ng/mL, patients with levels <20 ng/mL were more likely to develop CDI (OR 2.27, 95% CI 1.16–4.44). The mean plasma 25 (OH)D in patients with CDI who subsequently died was significantly lower (12.8 ± 8.1 ng/mL) compared to those who were alive at the end of follow-up (24.3 ± 13.2 ng/mL) (P = 0.01).

Conclusions

Higher plasma calcifediol [25(OH)D] is associated with reduced risk of *C. difficile* infection in patients with IBD. Further studies of therapeutic supplementation of vitamin D in patients with inflammatory bowel disease and *C. difficile* infection may be warranted.

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INTRODUCTION

Clostridium difficile infection (CDI) is an important cause of morbidity and mortality in patients with inflammatory bowel diseases [IBD; Crohn's disease (CD), ulcerative colitis (UC)].¹⁻⁵ It is associated with four-fold excess mortality and increased need for hospitalisation, emergency room visits and colectomy up to 5 years after the initial episode.^{3, 5–7} Both age and co-morbidity are recognised risk factors for CDI. However, compared to non-IBD patients with CDI, CDI-IBD patients are often younger and have fewer pre-disposing non-IBD related co-morbidities.^{5, 8} Furthermore, extrinsic risk factors identified in the general population such as antibiotic or proton pump inhibitor (PPI) use are less frequently reported in CDI-IBD patients. This suggests that other mechanisms may exist to explain the association between the two conditions.^{1, 9}

There is growing interest in the immunological role of vitamin D beyond its well-recognised effects on bone and mineral metabolism.¹⁰⁻¹⁴ Mice deficient in vitamin D or with knockout of the vitamin D receptor (VDR) demonstrate greater susceptibility to chemical models of colitis that can be ameliorated through administration of 1,25-dihydroxy cholecalciferol [1,25(OH)₂D₃].^{12, 15} An emerging body of literature supports a role for vitamin D deficiency in the pathogenesis¹⁶ as well as natural history of IBD^{13, 17, 18} through its effect on innate immunity. Given the important role of innate immunity in anti-microbial defence and the effect of vitamin D on pathogen sensing and other innate immune responses,^{10–12, 15} it is conceivable that vitamin D deficiency may play a role in susceptibility to CDI. Indeed it was recently demonstrated that cathelicidin, an anti-microbial peptide whose production is stimulated by vitamin D, inhibits the effect of C. difficile toxin.¹⁹ Furthermore, a prior study from our group examining genetic risk factors for CDI demonstrated an association with a single nucleotide polymorphism (SNP) on chromosome 7 associated with CREB5, a member of the cyclic AMP responsive element-binding protein family associated with expression of the cathelicidin promoter sequence.^{20, 21}

Consequently, we performed this study to examine whether plasma calcifediol, 25-hydroxy vitamin D [25 (OH)D] is associated with risk of subsequent CDI in a large multi-institutional cohort of patients with CD and UC. Identifying such an association will provide important insights into pathogenesis of CDI as well as suggest a role for vitamin D in the treatment of CDI.

METHODS

Study population

The data source for our study was a multi-institutional cohort of patients with established CD or UC. The establishment of our cohort has been described in previous publications.^{6, 17, 22, 23} In brief, we utilised electronic medical record (EMR) data from patients seeking care at one of two tertiary referral hospitals, Massachusetts General Hospital and Brigham and Women's Hospital, in the greater Boston metropolitan area serving a population of over three million people. All patients with an International Classification of Diseases, 9th edition, Clinical Modification (ICD-9-CM) code for CD (555.x) or UC (556.x) were identified from the EMR (n = 24 182). A classification algorithm was developed using codified data or narrative free text concepts identified through natural language processing. Our final algorithm had high specificity and positive predictive value of 97%, and yielded a cohort of 5506 patients who were classified as having CD and 5522 classified as having UC.²²

Variables and outcomes

This study included 3188 patients who had at least one measured plasma 25(OH)D as part of their routine clinical care. As described previously, patients with measured plasma 25(OH)D were similar in age, but more likely to be female, use immunosuppressive therapy or require hospitalisation for their IBD.^{17, 23} Plasma 25(OH)D was measured using radioimmunoassay prior to 2008 and high-performance liquid chromatography with mass spectrometry since then with similar ranges for normal values across both time periods and acceptable coefficients of variance (7.5–12.5%).

Our primary outcome of interest was the development of CDI identified through the presence of the appropriate ICD-9-CM code (008.45). The ICD-9-CM code for CDI has been validated previously and extensively used to define burden of disease and secular trends in infection.^{6, 24–26} Plasma 25(OH)D values measured after the diagnosis of *C. difficile* were excluded from the analysis.

Information was obtained on age at entry into our cohort, gender, race and co-morbidity (prior to CDI or end of follow-up) assessed using the widely used and validated Charlson co-morbidity index.²⁷ The type of IBD as well as use of immunosuppressive or corticosteroids for treatment of disease was ascertained using the electronic prescription function of our EMR. Corticosteroid use was defined as a prescription within 90 days prior to the diagnosis of CDI or the end of follow-up.

We also assessed the use of antibiotics, statins and proton pump inhibitors within 90 days prior to development of CDI or end of follow-up, as well as hospitalisation within 90 days prior to development of CDI.

Statistical analysis

All analysis was performed using Stata SE 12.1 (Stata-Corp, College Station, TX, USA). Continuous variables were summarised using means and standard deviations and compared using *t*-tests, while categorical variables were expressed as proportions and compared using the χ^2 tests with Fisher's exact modification when appropriate. Plasma 25(OH)D was modelled as a continuous variable in our primary analysis. In secondary analyses, it was model as a dichotomous (≥20 ng/mL; <20 ng/mL) or a stratified (<20 ng/mL, 20–29.9 ng/mL, \geq 30 ng/mL) variable. Univariate logistic regression with development of CDI as the outcome was performed, and variables significant in the univariate analysis at P < 0.10 were selected for inclusion in the final multivariate model where a two-sided P < 0.05 indicated independent statistical significance. To examine if the effect of vitamin D was mediated through its association with disease severity, we additionally adjusted for C-reactive protein or erythrocyte sedimentation rates where available. To account for the fact that patients who have their vitamin D level measured may be sicker than those who do not have an available vitamin D measure, we additionally constructed a propensity score estimating the likelihood a plasma 25(OH)D was obtained. Though our study population included only patients with at least one measured vitamin D level, we additionally adjusted for this propensity score in a sensitivity analysis to examine the robustness of our results.

Ethical approval was obtained from the Institutional Review Board of Partners Healthcare.

RESULTS

Our study included 3188 patients with CD or UC who had at least one measured plasma 25(OH)D among whom 35 patients developed *C. difficile* infection (CDI). Table 1 presents the characteristics of included patients with and without a history of CDI. Patients who developed CDI were older (mean age 60.5 years vs. 48.7 years) and more likely to have a Charlson co-morbidity index \geq 3. There was no difference in race, gender or IBD type between the two groups. Patients with IBD-CDI nearly universally had a history of hospitalisation within 90 days prior to *C. difficile* infection or had

Table 1 Characteristics of patients with inflammatory
bowel disease, stratified by diagnosis of Clostridium
difficile infection

Characteristic	With C. difficile infection (n = 35) %	Without C. difficile infection (n = 3153) %	P-value
Mean age (s.d.) (in years)	60.5 (16.9)	48.7 (18.0)	0.0001
Female	63	61	0.80
Nonwhite	11	13	0.82
Charlson co-morbidity			
0–2	8	47	< 0.001
≥3	94	53	
IBD type			
Ulcerative colitis	51	45	0.47
Crohn's disease	49	55	
Recent hospitalisation or surgery*	95	55	0.009
Antibiotic use*	95	75	0.009
Statin*	31	17	0.02
Steroids*	29	53	0.003
Proton pump inhibitor*	63	48	0.08
Immunosuppressive therapy	20	7	0.003
Mean plasma 25(OH)D (s.d.) (in ng/mL)	20.4 (12.8)	27.1 (12.7)	0.002

* Within 90 days of C. difficile infection for cases.

used antibiotic therapy. Immunosuppression (immunomodulator or anti-TNF biologics) was more common among those with CDI, while recent prescription of steroids was less frequent in those with CDI. The mean plasma 25(OH)D level was significantly lower in patients who developed CDI (20.4 ng/mL) compared to non-CDI-IBD patients (27.1 ng/mL) (P = 0.002) (Figure 1).





Examining vitamin D status as a dichotomous variable, levels below 20 ng/mL were associated with a two-fold increase in risk of CDI (Odds ratio(OR) 2.27, 95% confidence interval (CI) 1.16–4.44). The median interval between the measured plasma 25(OH)D and CDI in the case population was 228 days (interquartile range (IQR) 56–752 days).

We performed multivariate analysis examining predictors of CDI (Table 2). Plasma 25(OH)D level was an independent predictor of CDI after adjusting for potential confounders. Each 1 ng/mL increase in plasma 25 (OH)D was associated with a 4% reduction in risk of CDI (OR 0.96, 95% CI 0.93–0.99, P = 0.046). Greater co-morbidity, recent hospitalisation and immunosuppressive use were also associated with increased risk of CDI while steroid use was inversely associated with disease risk. Antibiotic proton pump inhibitor or statin use was not associated with risk of CDI in our cohort. Adjusting for a propensity score estimating likelihood of measuring plasma 25(OH)D did not alter our estimates.

One-quarter of patients with CDI-IBD (26%) subsequently underwent surgery, and one-third died during follow-up (34%) compared to corresponding rates of 16% (P = 0.13) and 5% (P < 0.001) among those without CDI. The risk of surgery was not different by vitamin D status. However, the mean plasma 25(OH)D in patients with CDI who subsequently died was significantly lower (12.8 ± 8.1 ng/mL) compared to those who were alive at the end of follow-up (24.3 ± 13.2 ng/mL) (P = 0.01) (Figure 2).

Table 2 | Multivariate analysis of predictors of

Clostridium difficile infection			
Characteristic	Adjusted odds ratio*	95% confidence interval	
Charlson co-morbidity	/		
0–2	1.0		
≥3	6.06	1.34 – 27.47	
Recent surgery or hos	spitalisation		
No	1.0		
Yes	8.84	1.98–39.46	
Steroid use			
No	1.0		
Yes	0.16	0.07-0.36	
Immunosuppressive u	se		
No	1.0		
Yes	3.82	1.46–9.99	
Serum 25(OH)D	0.96	0.93–0.99	

* Additionally adjusted for age, gender, race, season of measurement, IBD type, antibiotic use within 90 days and duration of follow-up.



Figure 2 | Plasma 25(OH)D and long-term mortality in IBD patients with *Clostridium difficile* infection.

DISCUSSION

Clostridium difficile infection is an important source of morbidity in patients with IBD.^{1–5} The association between IBD and CDI could be related to co-occurrence of risk factors; however, this does not adequately explain disease risk in a large proportion of patients.^{1, 4} Using a large multi-institutional IBD cohort, we demonstrate that higher vitamin D levels were independently associated with a reduced risk for CDI in patients with IBD. Secondly, IBD-CDI patients with low vitamin D levels had higher mortality when compared to those with sufficient levels.

Our findings intriguingly suggest a role for vitamin D in the pathogenesis of CDI, particularly in patients with IBD. Several pieces of experimental evidence support our results. Cathelicidin is a key anti-microbial peptide in mammals that is found at sites of inflammation and is involved in host defence against bacteria and other pathogens.²⁰ 1,25(OH)₂D₃ regulates its production by binding to the vitamin D receptor (VDR) and subsequently through the vitamin D response element (VDRE) at the cathelicidin promoter sequence, thereby regulating its production.²⁸ Cathelicidin is expressed widely in neutrophils, natural killer cells, keratinocytes, lymphocytes, dendritic cells, macrophages, as well as intestinal epithelium and has anti-bacterial activity against a spectrum of bacterial pathogens.^{20, 29} Intracolonic administration of the mouse cathelicidin is associated with amelioration of colitis.³⁰ In an elegant study by Hing et al., wild-type and cathelicidin-deficient mice were treated with antibiotics and infected orally with C. difficile. Exogenously administered cathelicidin was associated with reduced colonic tumour necrosis factor and myeloperoxidase levels and reduced histological damage in both the colon and the ileum.¹⁹ In a previous study, our group demonstrated an association between a SNP on chromosome 7 associated with the gene CREB5 and increased risk for CDI in UC patients.²¹ The CREB protein competes for binding to the cathelicidin promoter sequence and regulates its expression. In an earlier study, human alpha-defensins inhibited the effect of C. difficile toxin B³¹; adequate levels of 25(OH)D are essential for production of human defensins, further supporting a mechanistic association between 25(OH)D and CDI. Vitamin D and cathelicidin have also been associated with risk of other infections including tuberculosis, influenza A, hepatitis C and Helicobacter pylori.²⁹ Low levels of cathelicidin were associated with higher infection related mortality in patients on haemodialysis.³²

A few recent studies have examined this association between vitamin D levels and CDI in non-IBD cohorts.^{33–36} In a study from the Veterans affairs population, Youssef *et al.* identified a five-fold increase in risk of health care costs associated with CDI in individuals who were deficient in vitamin D compared to those with sufficient levels.³³ Wang *et al.* similarly demonstrated that individuals with CDI who were deficient in vitamin D were less likely to resolve infection,³⁶ consistent with data from van der Wilden *et al.* who demonstrated an inverse correlation between severity of CDI and plasma vitamin D levels.³⁵ Only one study previously examined vitamin D levels as a risk factor for hospital acquired CDI and identified an increase in risk of CDI in individuals with plasma 25(OH)D levels below 20 ng/mL.³⁴

Other independent predictors of CDI in our cohort were higher burden of co-morbid illness and immunosuppressive therapy consistent with prior studies.^{1, 5, 8, 37} Whether immunosuppressive therapy itself confers risk or is a marker for more active colonic disease³⁸ which may be associated with increased risk for CDI remains unclear. Adjusting for C-reactive protein levels as a marker of inflammation did not influence the magnitude of association between immunosuppression and CDI suggesting that there may indeed be an elevated risk associated with therapy. Interestingly, we observed an inverse association between corticosteroid use and CDI which is in contrast to prior data.^{37, 39} This may in part be due to the overall greater severity of disease in our cohort with higher frequency of immunosuppression than in some prior studies. As well, use of corticosteroids tends to be intermittent and of varying duration; consequently electronic prescription dates may be inadequate markers of dose and duration of use. Consistent with known epidemiology of CDI, recent hospitalisation or surgery remains one of the strongest risk factors for CDI. We observed no association between use of antibiotics, proton pump inhibitors or statin and risk of CDI. While studies in non-IBD patients have demonstrated the former two to be risk factors^{40–42} and the latter to be inversely associated with CDI risk,^{43, 44} data from IBD cohorts have not consistently demonstrated independent associations with medication use and risk of CDI,^{1, 9} and our findings confirm this lack of association.

There are a few implications to our findings. In IBD patients, prior studies have demonstrated low predicted plasma 25(OH) vitamin D levels to be associated with increased risk for CD,16 greater likelihood of IBD related hospitalisations, surgery^{13, 17, 45} and increased risk of malignancy, particularly colorectal cancer.²³ The present study explores another immunological consequence of vitamin D deficiency in patients with IBD and suggests that low vitamin D levels may be associated with an increased risk of CDI, an effect possibly mediated through reduced cathelicidin production. A study in patients with eczema demonstrated that high-dose oral vitamin D3 is associated with a significant increase in plasma cathelicidin levels,⁴⁶ suggesting that there is merit in future studies examining the role of vitamin D supplementation as an adjunct therapy in CDI-IBD. There is also an important need for studies examining the role of vitamin D in the pathogenesis of CDI in non-IBD cohorts.

There are several limitations to our study. First, the number of patients with a measured plasma 25(OH)D level who subsequently developed CDI was small. Though this is the first and largest study to date examining this association, it is important to replicate this association in additional cohorts. Second, as plasma 25 (OH)D was measured as part of routine clinical care, it was not available for all patients. Those with available 25(OH)D levels differed from those without any 25 (OH)D available. Third, as our cohorts were based at a tertiary referral centre, it is possible that there exists a bias towards more severe disease. However as both the CDI-IBD cases and the non-CDI-IBD controls were drawn from the same pool, it is unlikely that the bias differentially affects our association. Fourth, it is possible that sicker patients are at higher risk for CDI and as well for vitamin D deficiency owing to reduced physical activity and time spent outdoors. However, that our findings were robust to adjustment for immunosuppressive and steroid use, recent hospitalisation, surgery and co-morbidity suggest that this confounding is unlikely to explain our association. Fifth, the median interval between measured vitamin D and CDI was 228 days. It is possible that in the interim, some individuals who were identified to be deficient received vitamin D supplementation. However, such nondifferential misclassification of exposure would bias results towards the null making our estimates more conservative. Finally, it is possible that some patients with CDI were misclassified as controls. However, such misclassification would bias the effect towards the null, making our results a conservative estimate.

In conclusion, we demonstrate that higher vitamin D levels were associated with a reduced risk of CDI in patients with IBD. This suggests a potential role for vitamin D in the pathogenesis of CDI. As well, it raises interesting possibilities of whether vitamin D may have a role as adjunct therapy for CDI. Further studies in IBD and non-IBD cohorts are essential to replicate our findings, investigate the mechanisms of this association and its implications for patient care.

AUTHORSHIP

Guarantor of the article: A. N. Ananthakrishnan.

Author contributions: Ananthakrishnan: Study concept, Study design, Preliminary draft of the manuscript. Ananthakrishnan, Gainer, Cagan, Cai, Cheng, Churchill, Kohane, Shaw, Liao, Szolovits, Murphy: Data Collection. Ananthakrishnan, Cai, Cheng: Analysis. All authors approved the final version of the manuscript.

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