The Role of Vitamin D in Anti-Tumor Necrosis Factor-Alpha-Induced Response in Patients with Inflammatory Bowel Disease

By

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

In

Experimental Medicine

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ABSTRACT

Vitamin D is an important immunomodulator of the immune system and has been suggested to play a role in the pathogenesis of inflammatory bowel disease (IBD). Drugs targeting TNF-alpha are effective IBD therapies, and vitamin D has been demonstrated to suppress TNF-alpha as well as work synergistically with infliximab to reduce TNF-alpha *in vitro*. As a result, vitamin D may play a role in anti-TNF-induced response.

The objectives of this study were to first compare the proportion of patients who achieved a clinical response in the normal vitamin D group to the proportion of patients who achieved a clinical response in the low vitamin D group at week 14; and to secondly compare clinical response rates at week 22, after the low vitamin D group was supplemented at week 14. Secondary outcomes included assessing clinical remission, C-reactive protein normalization, cytokine responses, health related quality of life, and depression at these time points.

Adult Crohn's disease and ulcerative colitis patients initiating anti-TNF therapy were invited to participate. Prior to starting anti-TNF therapy and at week 14, blood samples were collected to measure serum vitamin D, C-reactive protein, and cytokines levels and questionnaires were administered to assess clinical disease activity, depression, and quality of life. Patients low in vitamin D (serum 25(OH)D levels <75 nmol/L) were then administered a high dose (250,000-500,000 IU) of vitamin D intramuscularly within 2 weeks of their week 14-dose. Patients with normal vitamin D levels were not supplemented. Measurements of vitamin D, C-reactive protein, cytokines, clinical disease activity, depression, and quality of

life were repeated 8 weeks later, prior to the patient's week 22- dose. Clinical response at week 14 and week 22 was defined as a decrease of ≥ 3 points in the clinical disease activity scores from baseline.

The proportion of patients who clinically responded at week 14 was similar between the two vitamin D groups (67% (14/21) vs. 65% (15/23), p=0.92). However, after stratifying by disease severity, there was a clinically significant higher proportion of patients in the low vitamin D group who responded at week 14 compared to the normal vitamin D group, if patients had severe disease (79% (11/14) vs. 53% (9/17), p=0.14). On the contrary, there was a trend to a higher proportion of patients in the normal vitamin D group who responded at week 14 compared to the low vitamin D group, if patients had non-severe disease (100% (4/4) vs. 44% (4/9), p=0.11). Clinical response results at week 22 were similar. Patients with low vitamin D levels and severe disease had higher serum levels of TNF-alpha, IL-6, and IL-1beta at baseline compared to patients with normal vitamin D levels and severe disease. By week 14 and week 22, cytokine levels were similar. Quality of life scores paralleled improvement in disease activity, and patients with low vitamin D levels had more cognitive depressive symptoms at the start of therapy.

In conclusion, the inflammatory responses in patients with severe disease and low vitamin D levels are effectively treated with infliximab and adalimumab, and it may be that having inadequate levels of vitamin D before initiating anti-TNF therapy increases IBD patients' sensitivity to this drug.

PREFACE

This thesis is original work by Krista Reich. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, “The Role of Vitamin D in Anti-Tumor Necrosis Factor-Alpha-Induced Response in Patients with Inflammatory Bowel Disease”, No. Pro00031844, August 2012

No part of this thesis has been previously published.

AKNOWLEDGEMENTS

I would like to thank my committee members for guiding me through this thesis. They took the time to teach and support me, while pushing me to excel to the next level. There were many challenges, but their encouragement and belief in me kept me moving forward.

I would like to thank the gastroenterologists who allowed me to contact their patients for study participation.

I would like to thank the infliximab infusion clinic nurses for their patience and volunteering their time to collect patient samples for this study. I would also like to thank the IBD nurses for all of their contributions that made this study move smoothly.

It was great to work with the members of the Fedorak and Madsen lab. Everyone was very helpful and kind. I would like to thank them for their assistance in patient recruitment, data collection, and cytokine analysis.

I am especially thankful for all of the support from my supervisor Dr. Karen Kroeker. She has been a great mentor, believing in me and building my confidence. She has taught me life skills that I will always carry with me.

I would also like to thank Dr. Richard Fedorak for his endless support and guidance.

I would like to acknowledge my parents and friends who show me unconditional love.

Finally, I would like to thank all the study participants. It was great getting know many different people over the 22 weeks of follow up. It meant a lot to me that they were interested in the study and were excited to be a part of my project.

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List of Symbols and Abbreviations

IBD – inflammatory bowel disease

VDR – vitamin D receptor

TNF-alpha – Tumor necrosis factor-alpha

Th1/Th2 – T helper Type 1 and 2 lymphocytes

5-ASA – 5 aminosalicylic acid

IL – interleukin

LPS – lipopolysaccharide

HRQoL – health related quality of life

SIBDQ – short inflammatory bowel disease questionnaire

BDI-II – Beck Depression Inventory-II

CD - Crohn's disease

UC – ulcerative colitis

n – number of subjects or sample size

IQR – interquartile range

Ref rang – reference range of what levels are defined as 'normal'

SPSS - Statistical Package for the Social Sciences

vs. – versus

β – beta

α – alpha

> - greater than

< - less than

≥ - greater than or equal to

≤ - less than or equal to

1 Introduction

1.1 Inflammatory Bowel Disease

1.1.1 Inflammatory Bowel Disease: Crohn's Disease and Ulcerative Colitis

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the intestine that causes abdominal pain, diarrhea, and weight loss. It includes two forms, Crohn's disease (CD) and ulcerative colitis (UC). Crohn's disease is characterized by inflammation of any part of the intestine, with skip lesions where the inflammation does not affect regions of the intestine and severely affects other regions. Ulcerative colitis is characterized by inflammation restricted to the rectum and may affect the colon in an uninterrupted pattern.[1] The disease etiology is not completely understood and the incidence is increasing world wide.[2] Evidence suggests that IBD results from an inappropriate immune response to the body's natural intestinal bacteria, which activates the gastrointestinal immune system; however, it is very complex as genetic and environmental interactions play a role in disease susceptibility and progression.[1,2]

1.1.2 Immune Defects in IBD

The immune system consists of innate immune responses and adaptive immune responses. The innate immune response is the body's first line of defense, which quickly and non-specifically responds to pathogens.[1,3] This includes immune cells and physical barriers such as the intestinal mucosa.[4] The adaptive immune system mediates a very specific immune response, which develops an immunological memory over time.[1,3] There is tight cross-regulation between these two arms of the immune system; as a result, a shift in this balance can lead to a dysregulated immune response and excessive inflammation, leading to IBD.[4]

Innate immune defects in IBD include epithelial cell damage and excessive inflammatory responses from macrophages, monocytes, and dendritic cells.[4] Epithelial damage can result in entry of bacteria from the intestinal lumen into circulation and increase contact between antigens and immune cells in the submucosa.[1] This results in activation of the innate immune system by pattern recognition receptors followed by antigen presenting cells and stimulation of pro-inflammatory T helper (Th) Type 1 lymphocyte responses of the adaptive immune response.[3,5] Consequently, dysregulation of the innate immune response results in over-stimulation of the adaptive immune system. IBD is mediated by overly aggressive activity of effector T cells, leading to large amounts of proinflammatory cytokines and inflammation that leads to tissue damage.[4] In that there is no cure for IBD, medical therapy remains the mainstay treatment for achieving and maintaining remission.[2]

1.2 Anti-Tumor Necrosis Factor in the Treatment of IBD

Tumor necrosis factor-alpha (TNF-alpha) is a central cytokine in the pathogenesis of IBD and is produced by multiple cells, including lamina propria mononuclear cells, macrophages, and T cells.[6] It has been shown to be elevated in the stool, mucosa, and blood of patients with IBD.[7,8,9] It has many pro-inflammatory effects, from inducing cell death of Paneth cells to activating macrophages and effector T cells.[6] Specifically, activation of TNF receptor 2 induces activation of the transcription factor nuclear factor κB (NF κB) and upregulates pro-inflammatory cytokines such as interleukin-6 (IL-6).[10] Additionally, it increases T cell proliferation and secretion of more inflammatory cytokines, including TNF-alpha and IFN-gamma.[5] As a result, anti-TNF therapies have been developed to target this cytokine. These are antibodies that neutralize both soluble and membrane-bound TNF-alpha.[6] Their therapeutic benefits have been attributed to multiple effects, including high affinity binding to TNF-alpha thereby neutralizing its biologic activity and inducing T cells apoptosis.[5,6] Moreover, by decreasing T cell activation and proliferation, it reduces cytokine secretion from these

cells. Anti-TNF drugs have also been demonstrated to stimulate protective regulatory T cells thereby maintaining immune homeostasis.[5]

Infliximab and adalimumab are two anti-TNF therapies that have been approved in Canada for use in Crohn's disease and ulcerative colitis. Infliximab is a chimeric mouse immunoglobulin G1 monoclonal antibody to TNF-alpha and is given as a 5 mg/kg intravenous infusion.[11,12,13] Adalimumab is a subcutaneously administered recombinant fully human immunoglobulin G1 monoclonal antibody that binds with high affinity and specificity to TNF-alpha.[14] Both of these drugs have been shown to be effective induction and maintenance therapies for patients with moderate-severe IBD.[11,13,14] There are two phases to the dosing of infliximab and adalimumab: the induction phase and the maintenance phase. Infliximab is administered at week 0, 2 and 6 as an induction regimen, and then every 8 weeks as a maintenance regimen.[11,13] Adalimumab is administered as an induction regimen at a dose of 160 mg at week 0 and 80 mg at week 2 and then every 2 weeks at 40 mg as a maintenance regimen; the does is not weight-based.[14]

Conventionally, treatment of IBD takes a sequential step-up approach wherein drug therapy is intensified as the disease worsens and there is no response. As a result, patients will start with 5-aminosalycyclic acid (5-ASA) then progress to corticosteroids, immunosuppressants (azathiopurine/sulfasalazine), anti-TNF therapy, and lastly, surgery.[15] The invasiveness of surgery and the severity of its potential side effects can significantly affect patients' lives, making this option significantly less attractive for most patients.[16] Surgery rates remain relatively high, as it has been recently reported that the 5-year cumulative risk of resection in Crohn's disease was 24.6% and the 5-year-cumulitve risk of colectomy in ulcerative colitis was 10.4%.[17] As a result, patients remain at risk for disease relapse[18,19] and in order to avoid surgery, the goal is to optimize IBD outcomes by improving response rates to anti-TNF therapies.

1.3 Vitamin D Deficiency and IBD

Epidemiological evidence supports a role for the deficiency of an environmental factor, vitamin D, in the pathogenesis of IBD. This is demonstrated by the 'north-south' gradient in the risk of Crohn's disease and ulcerative colitis, likely explained by the variation in sun exposure, a major determinant of vitamin D levels.[20,21] Vitamin D deficiency has been well described in IBD patients from all over the world[22], with reports of up to 90% of IBD patients having a vitamin D level < 75 nmol/L.[23] Furthermore, higher vitamin D plasma levels, predicted on the basis of a validate regression model in the Nurses' Health Study, have been shown to be associated with a lower incidence of Crohn's disease and ulcerative colitis[24]; as a result, obtaining and maintaining optimal vitamin D levels are important.

Vitamin D is an important immunomodulator of both the innate and adaptive immune systems.[25] Immune cells express vitamin D receptors (VDRs) and the enzymes necessary to convert vitamin D into its active form, $1,25(OH)_2D3$; as a result, locally produced $1,25(OH)_2D3$ can exert specific autocrine and paracrine effects.[26] $1,25(OH)_2D3$ can modulate the adaptive immune responses by altering actions of activated T and B cells, and it can modulate the innate immune responses by regulating macrophage and dendritic cell activity.[26] As a result, these findings sparked investigation into the immunomodulatory role of vitamin D in the pathogenesis of autoimmune diseases such as IBD.

1.4 Immunomodulatory Effects of Vitamin D

1.4.1 Vitamin D and the Adaptive Immune System

Cytokines are proteins produced by immune cells and help determine the type of immune response.[26] Many cytokines take part in the inflammatory response of the mucosa and play a role in the pathogenesis and disease activity of IBD. Th1 and Th2 T cell responses have been demonstrated to characterize the inflammatory response in

IBD, and 1,25$(OH)_2$D3 has anti-inflammatory effects by mediating the Th1-Th2 balance in favour of Th2 cell development. Th2 cells produce anti-inflammatory IL-4, IL-5, and IL-13, while Th1 cells produce pro-inflammatory IFN-γ and lymphotoxin.[26,27] As a result, vitamin D regulates cytokine production to limit inflammatory tissue damage and skews the immune responses towards a Th2 phenotype, which fights extracellular infections.

Vitamin D also targets Th17 cells and suppresses their ability to produce IL-17, a pro-inflammatory cytokine, thereby reducing inflammatory tissue damage. 1,25$(OH)_2$D3 also increases the development of T regulatory (T reg) cells, which have immunosuppressive properties by depressing the proliferation of other CD4+ T cells.[25] Vitamin D in combination with another immunosuppressive drug, dexamethasone, has been shown to increase IL-10 producing regulatory T cells to downregulate immune responses at sites of inflammation.[28]

Research supports the immunosuppressive effect of vitamin D due to its involvement in T-cell differentiation. The evidence shows that vitamin D plays a role in maintaining a balance between the inflammatory response of Th1/Th17 cells and the immunosuppressive response from Th2/Treg cells.

1.4.2 Vitamin D and the Innate Immune System

1.4.2.1 Vitamin D suppresses dendritic cell stimulation of Th1 responses

Dendritic cells (DCs) are antigen presenting cells (APCs) and are important in initiating Th1 cell development.[29] Vitamin D inhibits differentiation and maturation of human DCs and suppresses their production of IL-12. In that IL-12 stimulates Th1 cell development, vitamin D acts as an immunosupressor by indirectly inhibiting Th1 proliferation and its corresponding inflammatory responses.[26,27] DCs still mature, therefore, normal immune responses still occur; as a result, vitamin D regulates the immune system to prevent responses that may lead to pathological effects.[25]

1.4.2.2 Anti-microbial activity of vitamin D

Vitamin D also stimulates anti-bacterial activity. Autophagy, an important mechanism for eliminating pathogens by antibacterial proteins, is triggered in human monocytes by locally produced 1,25$(OH)_2$D3.[22,30] Toll-like receptors (TLR) expressed on monocytes recognize pathogens, and when these receptors are stimulated, the expression of VDRs and CYP27B1, the gene encoding for the enzyme, 1α-hydroxylase, that converts vitamin D into 1,25$(OH)_2$D is upregulated.[25,30] Locally produced 1,25$(OH)_2$D3 acts in an autocrine fashion to induces expression of the cathelicidin antimicrobial peptide (CAMP) gene (LL-37) in monocytes, producing a protein that enhances intracellular killing of bacteria.[31] Furthermore, nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a pattern recognition receptor, is important for stimulating anti-bacterial activity once activated by bacterial products. Bacterial activation of NOD2 stimulates transcription factor NF-κB, which induces gene expression for anti-microbial peptide defensin β2 (DEFβ2). Vitamin D induces expression of NOD2 in multiple cells, thereby increasing the sensitivity of these cells to bacterial products and enhancing NOD2 induced DEFB2 anti-bacterial activity.[32]

1.4.2.3 Vitamin D suppresses bacterial-stimulated proinflammatory responses

As a part of the innate immune system, stimulated macrophages release proinflammatory cytokines and chemokines, to protect the body from pathogens.[33] Proinflammatory cytokines are positive for host defense, but overproduction leads to unresolved inflammation.[34] Vitamin D treatment has been demonstrated to reduce these pro-inflammatory responses. Lipopolysaccaride (LPS) is a component of the Gram-negative bacterial wall, which induces monocytes/macrophages to produce cytokines. 1,25$(OH)_2$D3 treatment with LPS-stimulated human blood monocytes inhibited the release of IL-1alpha, IL-6, and TNF-alpha.[35] Furthermore, LPS binds to Toll-like receptor 4 (TLR4) on monocytes to mediate activation of MAPK, which regulate proinflammatory cytokine production, including IL-6 and TNF-alpha.[34] Treatment with 25(OH)D3 has been shown to inhibit LPS-induced IL-6 and TNF-alpha production in peripheral blood

mononuclear cells (PBMC) of healthy donors via upregulation of MKP-1 (MAPK phosphatase-1); MKP-1 switches off cytokine production in monocytes/macrophages after inflammatory stimuli.[34] LPS exposure could occur in the gut of IBD patients; as a result, vitamin D may be an important regulator of pro-inflammatory responses in IBD. Furthermore, vitamin D receptor signalling has also been shown to play a very important part in immune-regulation as there is a dysregulated and oversustained innate immune response in macrophages under attenuated VDR signalling.[33] Overall, vitamin D is an immunomodulator that strengthens the antimicrobial roles of the innate immune response and depresses the inflammatory adaptive immune reaction.

1.5 Protective Immunomodulatory Effects of Vitamin D in IBD

Vitamin D and its VDR have been shown to have an important role in animal models of colitis. Mice raised on vitamin D-deficient chow had significantly lower 25(OH)D levels and markedly worse dextran-sodium-sulfate (DSS)-induced colitis.[36] Furthermore, VDR knock-out mice treated with DSS to induce colitis demonstrated markedly elevated levels of a number of tissue pro-inflammatory cytokines, including TNF-alpha, IL-12p70, and INF-gamma and demonstrated worse intestinal injury. In contrast, oral vitamin D supplementation in these mice led to higher levels of the anti-inflammatory cytokine IL-10 and improved intestinal injury.[37]

Studies have suggested that vitamin D deficiency and impairment in its signaling pathways are contributing factors in the pathogenesis of IBD. Vitamin D deficiency may lead to IBD by changing vitamin D receptor signaling in autophagy homeostasis, resulting in increased TNF-alpha-induced autophagy.[38] Additionally, *in vitro* studies have demonstrated a molecular link between vitamin D deficiency and NOD2 function. NOD2 deficiency due to mutations in its gene has been linked to the pathogenesis of Crohn's disease, and it has been shown that 1,25$(OH)_2$D3 signaling induces NOD2 expression in human intestinal epithelial cells, giving support to the idea that vitamin D deficiency plays a contributing role in the pathogenesis of Crohn's disease.[32]

Vitamin D suppresses the secretion of TNF-alpha, a central cytokine in the pathogenesis of IBD. A vitamin D analogue has been shown to work synergistically with infliximab, an anti-TNF therapy used in the treatment of IBD, to reduce the cytokine TNF-alpha in human peripheral blood monocyte.[39] Additionally, vitamin D treatment in the colonic tissue of IL-10 knock-out mice has been shown to down-regulate TNF-alpha-associated genes in these mice.[40] As a result, vitamin D has been demonstrated to have a protective role against IBD as well as is an important immune-regulator of inflammatory responses that occur in IBD. Subsequently, this suggests that vitamin D has a role to play in the therapeutic management of IBD.

1.6 Vitamin D Deficiency and Disease Activity

Inflammation in IBD has been shown to be associated with vitamin D deficiency. Vitamin D levels have been shown to correlate negatively with disease activity assessed by the Harvey Bradshaw score[41,42,43], Crohn's disease activity index (CDAI) score[44], and the six-point partial Mayo index.[45] Furthermore, Blanck et al.[45] stratified ulcerative colitis patients based on their vitamin D levels as either vitamin D sufficient (>74 nmol/L), insufficient (50-74 nmol/L), and deficient (<50 nmol/L) and demonstrated a trend towards more active disease as vitamin D levels decreased. Therefore, there is a relationship between vitamin D levels and inflammation; however, the direction of this relationship is still unclear. A recent study examined the relationship between vitamin D status and markers of disease activity in IBD while correcting for the differences due to potential confounders.[46] Similar to the previous studies, serum 25(OH)D levels were inversely proportional to intestinal inflammation measured by fecal calprotectin; this was not influenced by sun light exposure, skin type, oral vitamin D intake, or malabsorption in Crohn's disease and ulcerative colitis. This was also demonstrated in a subgroup of Crohn's colitis and ulcerative colitis patients wherein inflamed mucosa was restricted to the colon and did not affect absorption of vitamin D.[46] Therefore this study supports the immunomodulatory effects of vitamin D in human IBD. Interestingly, there was no association between serological markers of inflammation (e.g., CRP, white cell

count, platelet count) and vitamin D status. Since this association was only demonstrated with disease activity measured by fecal calprotectin, a good indicator of intestinal inflammation, the authors concluded that serum vitamin D status influences local tissue inflammation, not systemic inflammation.[46] This supports the idea that disease activity may lead to lower vitamin D levels as immune cells in the intestine work to increase the production of $1,25(OH)_2D$. However, lower 25(OH)D3 levels may initially lead to lower $1,25(OH)_2D$ levels, exacerbating disease activity, and in response, lead to stimulation of $1,25(OH)_2D$ production.

Despite a high prevalence of vitamin D deficiency among IBD patients, serum vitamin D levels may not always be associated with disease activity. Hassan et al.[47] found no association between low vitamin D levels and increased disease activity in IBD patients. Vitamin D deficiency may also be explained by multiple other factors including the increased risk of intestinal malabsorption among the IBD population or inadequate sun exposure to sunlight either related to lifestyle or persistent symptoms of active disease restricting physical activity.[48] It has been demonstrated that Crohn's disease patients with quiescent disease have on average a 30% decrease in their ability to absorb vitamin D in comparison to normal subjects after supplemented with 50,000 IU of vitamin D2.[49] Furthermore, Suibhne et al.[23] report vitamin D deficiency to be common among Crohn's disease patients in clinical remission. Even in the summer, vitamin D deficiency among these patients continued to remain high (50%). As a result, the location of disease, disease activity, or prior resection may not be the only factors affecting vitamin D bioavailability.[49]

1.7 Vitamin D Supplementation Improves IBD Outcomes

Vitamin D supplementation has traditionally been recommended in patients with IBD for management of bone disease; however, there is now increasing evidence for the potential immunomodulatory effects of supplementation. The optimal level of 25-OH vitamin D for immunomodulatory effects is not known; however, Holick[50] (2007) has

reported that levels of 75 nmol/L or higher provide adequate substrate for 1α-hydoxylase in immune cells to locally produce 1,25-hydroxyvitamin D.

To date, there is only one randomized placebo-controlled study that has assessed the effectiveness of vitamin D supplementation in improving Crohn's disease outcomes. Compared to placebo, oral vitamin D supplementation of 1200 IU in adult patients with Crohn's disease in remission was shown to increase the 25-OH vitamin D levels and reduce the risk of relapse from 29% to 13% at 1 year (p=0.06).[51] Although this difference in relapse was not statistically significant, the difference is clinically meaningful and does warrant further study. Furthermore, the effects of active vitamin D (alfacacidiol) supplementation on disease activity were compared to non-active vitamin D (cholecalciferol) in Crohn's disease patients, and the active vitamin D treatment resulted in a significant decrease in CDAI scores and CRP levels. This difference was not maintained at 12 months; however, it had prominent short-term effects and may be due to improved immune responses.[52] As a result, active vitamin D may have additional improvements compared to the plain form. A third vitamin D supplementation study by Yang et al.[53] demonstrated clinical response in 78% of Crohn's disease patients after 24 weeks of supplementation. Patients were started with 1,000 IU/day of vitamin D3, and the dose was increased every two weeks by 1000 IU until serum 25(OH)D3 levels were above 40 ng/ml (100 nmol/L) or the patients were taking 5000 IU/day. After 24 weeks, the maximum dose of 5000 IU/day was required by 78% of patients and effectively raised serum 25(OH)D levels.[53] Although further investigation is required, vitamin D supplementation alone has shown clinical benefit in Crohn's disease patients.

Retrospective studies have also demonstrated a protective benefit of achieving normal vitamin D levels. The risk of IBD-related surgery has been demonstrated to increase In IBD patients who have low plasma 25(OH)D levels. Additionally, in Crohn's disease patients, if their vitamin D level was normalized, these patients were less likely to undergo surgery in comparison to patients who continued to maintain a low vitamin D level.[54] Zator et al.[55] also demonstrated a protective effect of vitamin D in IBD. They

demonstrated a significant association between earlier cessation of anti-TNF therapy in IBD patients who had insufficient vitamin D levels prior to initiation of anti-TNF therapy, suggesting vitamin D may be an important adjuvant treatment aiding in the maintenance of response to this therapy. These studies denote the importance of repleting and maintaining sufficient vitamin D levels in patients who have IBD, specifically above 30 ng/ml (75 nmol/L), to reduce the risk of flares and to maintain response to IBD-therapies.[54,55]

1.8 Vitamin D and Health-Related Quality of Life

Health-related quality of life (HRQoL) is a quantitative measurement of one's subjective perception of one's health state, emotionally and socially and is impaired among patients with IBD. IBD patients with active disease have lower HRQoL compared to patients in remission, with no difference between patients with Crohn's disease and ulcerative colitis.[56] Vitamin D deficiency in patients with IBD has also been shown to be associated with impaired quality of life. A retrospective study demonstrated that IBD patients with vitamin D deficiency were found to have significantly lower mean quality of life scores, assessed by the short inflammatory bowel disease questionnaire (SIBDQ), compared to patients who were not vitamin D deficient. However, after adjustment for disease activity, the association was no longer significant between vitamin D deficiency and lower HRQoL, but there was still a strong trend toward deficiency being independently associated with lower SIBDQ scores.[41] Therefore, vitamin D supplementation in vitamin D deficient IBD patients may be important in improving quality of life.

Few studies have examined the role of vitamin D supplementation in improving quality of life in IBD patients. A study by Yang et al.[53] showed promising results in vitamin D deficient Crohn's disease patients with oral vitamin D supplementation. Eighteen patients were supplemented with a maximum dose of 5000 IU/day of vitamin D3 for 12 weeks and quality of life was assessed by the inflammatory bowel disease questionnaire

(IBDQ), which measures disease-specific quality of life. Baseline IBDQ scores indicated poor quality of life, and after supplementation for 12 weeks, 56% of patients had improved IBDQ scores, which were maintained at 24 weeks. Disease activity (assessed by CDAI scores) and quality of life scores were inversely correlated at baseline and after supplementation, as well as serum vitamin D levels and change in levels after supplementation showed a positive correlation with quality of life scores.[53]

An additional study by Miheller et al.[52] assessed quality of life in Crohn's disease patients who were treated with either cholecalciferol (plain vitamin D) or alfacalcidiol (active vitamin D). There was significant improvement in quality of life scores measured by the short IBD questionnaire in the active vitamin D group at 6 weeks compared to the plain vitamin D group; however, by 12 months, there was no difference in scores between the two groups. This study supports the short-term effects of active vitamin D, but it fails to report how the scores at 12 months compared to the baseline scores. Therefore it is difficult to make a conclusion based on cholecalciferol supplementation. However, these studies demonstrate that quality of life and disease activity are related and vitamin D supplementation can improve both concurrently.

1.9 Vitamin D and Depression

Vitamin D deficiency has also been linked to depression and other mental health disorders. A recent systematic review supports the association between low vitamin D concentrations and depression.[57] The mechanism by which vitamin D may be associated with mental disorders is not clearly understood; however, there are vitamin D receptors in the hypothalamus, which may be important in neuroendocrine functioning.[58] Chronic medical illnesses, including IBD, are associated with higher rates of depression compared to healthy controls[59,60]; however, there are no studies examining the role of vitamin D supplementation in improving depressive symptoms in this population. Interestingly, there is new evidence showing that depression is associated with chronic low-grade inflammation. Indeed, cytokines have been shown to induce depressive-like

behaviors.[61] The modulatory effects of vitamin D on the immune system in reducing pro-inflammatory cytokines may play a key role in reducing depressive symptoms, specifically among the IBD population. The effects of oral supplementation in improving depressive symptoms have been controversial, with reports of vitamin D supplementation significantly improving depressive symptoms[62,63,64] and other reports showing no difference in depressive symptoms following vitamin D supplementation; however, low vitamin D levels were still shown to be associated with depressive symptoms.[65,66] These reports support a relationship between vitamin D and symptoms of depression; however, additional randomized clinical trials examining vitamin D supplementation in improving depressive states are needed, both using oral vitamin D and intramuscular vitamin D in an IBD population. It will be important to consider the dose, vitamin D status of the study group, and if these patients have depressive symptoms prior to supplementation.

Although current data in the IBD population is limited, vitamin D deficiency is linked with impaired quality of life and depression. Stronger evidence is required to support the positive impact that supplementation will have on these clinical outcomes in IBD patients. It will be important to consider that vitamin D deficiency is associated with increased disease activity in IBD patients[41] and that IBD patients with active disease have impaired quality of life and more depressive symtoms.[56] Therefore, it may be best to assess disease activity by examining both clinical disease activity assessment tools as well as health related quality of life and depression assessment questionnaires.

1.10 Study Purpose and Objectives

The purpose of this study is to evaluate the role of vitamin D levels and supplementation in inducing response to anti-TNF therapy. The relationship between vitamin D and immune system function is well supported, and there is increasing evidence suggesting vitamin D is an environmental factor influencing the course and severity of IBD. *In* vitro studies have suggested a synergistic role for vitamin D and infliximab in reducing

inflammation[39]; however, no studies to date have looked at the role of vitamin D in anti-TNF-induction of response in patients with IBD. Vitamin D supplementation has been shown in one study to reduce the risk of relapse[51]; however, stronger evidence is needed to support the effect of vitamin D in inducing and maintaining response in IBD patients. In that multiple factors contribute to the pathogenesis of IBD with increasing complexity as the disease progresses, strategies aimed at improving treatment of the disease are needed. Successful management will require combination therapies that target a number of pathways sequentially or concomitantly.[4]

1.11 Hypothesis

Patients with inflammatory bowel disease who have normal vitamin D levels have a superior clinical response to anti-TNF induction therapy than do those who have low vitamin D levels.

1.12 Specific Objectives

1.12.1 Primary Objectives

1. To determine if patients with low serum vitamin D levels prior to initiating therapy have a decreased clinical response to anti-TNF therapy at week 14 compared to patients with normal serum vitamin D levels
2. To determine if repletion of vitamin D in patients low in serum vitamin D results in an additional clinical response to anti-TNF therapy at week 22

1.12.2 Secondary Objectives

1. To reassess the primary objectives using clinical remission as the outcome
2. To compare C-reactive protein (CRP) levels, cytokine profiles, quality of life scores, and depression scores before initiation of anti-TNF therapy and 14 weeks after initiation of anti-TNF therapy between IBD patients with normal vitamin D levels and low vitamin D levels

3. To compare C-reactive protein (CRP), cytokine profiles, quality of life scores, and depression scores 22 weeks after initiation of anti-TNF therapy between IBD patients with normal vitamin D levels and IBD patients with low vitamin D levels 8 weeks after supplementation

2 Overview of Vitamin D Absorption and Metabolism

2.1 Vitamin D

Vitamin D or calciferol is a group of lipid soluble compounds with a four-ringed cholesterol backbone and consists of vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol).[67] Vitamin D3 is endogenously synthesized in the skin when sunlight and ultraviolet light converts 7-dehydrocholesterol into previtamin D3, which then rapidly converts into vitamin D3 (cholecalciferol).[50,68] Cutaneously produced vitamin D3 is released from the plasma membrane lipid bilayers of the dermis cells into extracellular space. Vitamin D binding proteins (DBPs) in the dermal capillary bed then transport vitamin D3 into the systemic circulation.[68,69] The precursor 7-dehydrocholeasterol is produced in large quantities in the skin; however, excess previtamin D3 or vitamin D3 in the skin is destroyed by sunlight, preventing intoxication from excess sunlight exposure. Vitamin D is also exogenously obtained from food and supplements; however, few foods naturally contain or are fortified with vitamin D.[50] Vitamin D3 is obtained from animals sources such as oily fish and eggs, or it can be manufactured by ultraviolet irradiation of 7-dehydrocholesterol from lanolin. Vitamin D2 (ergocalciferol) is synthetic and manufactured through ultraviolet irradiation of ergosterol from yeast; it is assumed to have the same biological activity in man as the 'natural' cholecalciferol (vitamin D3).[50] The only difference between vitamin D2 and D3 is the double bond between carbons 22 and 23 and the methyl group on carbon 24 in vitamin D2[70]; therefore, the "D" in vitamin D represents D2 and D3.[50]

2.2 Oral and Intramuscular Vitamin D Absorption

Dietary vitamin D (cholecalciferol or ergocalciferol) is absorbed in the gastrointestinal tract, specifically in the proximal small bowel.[67,68] Both vitamin D2 and vitamin D3 are relatively non-polar molecules and need to be solubilised into a bile-salt micellar solution in order to be absorbed into the aqueous phase. It is first emulsified into mixed micelles and then taken up by enterocytes. It diffuses through the unstirred-water layer, is then up taken by the brush-border membrane, and then transported out of the intestinal cell.[68] This is followed by incorporation into chylomicrons, which transport vitamin D into the venous circulation via the lymphatic system.[67,68] Vitamin D can also be administered intramuscularly, wherein cholecalciferol in sesame oil is released into the deep muscle tissue. A depot forms inside the muscle tissue acting as a repository and the vitamin D is gradually absorbed into the blood stream.[71]

2.3 Vitamin D Metabolism and Storage

Circulating vitamin D (calciferol) is transported by vitamin D binding proteins (DBPs) to the liver where it is converted to 25-hydroxycholecalciferol (25(OH)D) by 25-hydroxylase.[72,73] The dynamics of vitamin D storage and re-entry into circulation remain poorly understood; however, studies have shown that excess non-hydroxylated vitamin D (D3 or D2) and 25(OH)D are stored in adipose tissue and voluntary muscles for later use.[67,68,69] Beneficially, storage of vitamin D in adipose tissue prolongs its total-body half-life to approximately 2 months.[68,69,73] 25(OH)D3 is released to circulate in the blood,[69] where it is predominantly bound by DBPs and albumin, leaving little in the free form.[50,72] Circulating 25(OH)D then undergoes a second hydroxylation (1α-hydroxylated) in the mitochondria of the proximal tubules of the kidney to form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). Maintaining normal vitamin D levels is important as adequate levels of 25(OH)D must exist for it to be hydroxylated into $1,25(OH)_2D$ in the kidneys and extra-renal tissues.[68,70,73] $1,25(OH)_2D$ is now physiologically active and can act on its receptor to carry out many biological effects. $1,25(OH)_2D$ and 25(OH)D are then catabolized into

their inactive metabolites by 24-hydroxylase and excreted as calcitroic acid in the urine.[50]

The rate limiting enzyme in the metabolism of vitamin D is 1α-hydroxylase, which is tightly regulated by parathyroid hormone (PTH) and 1,25$(OH)_2$D3. PTH upregulates transcription of CYP27B1, the gene encoding for 1α-hydroxylase, which results in an increased production of 1,25$(OH)_2$D3 in the kidney. As a negative feedback mechanism, 1,25$(OH)_2$D3 suppress the transcription of PTH and CYP27B1, to decrease its production. Simultaneously, 1,25$(OH)_2$D3 induces 24-hydroxylase production.[50]

2.4 Assessment of Vitamin D Status

When assessing vitamin D absorption, serum concentrations of vitamin D (calciferol) are examined. Furthermore, when assessing the bioavailability of vitamin D, it is the amount of 25-hydroxyvitamin D (25(OH)D) that increases after administration of a dose of vitamin D (calciferol).[67] Moreover, when assessing vitamin D status, measuring 25(OH)D concentrations is clinically the most useful assessment. This is because it has a serum half life of approximately 3 weeks to 1 month, whereas 1,25$(OH)_2$D has a short circulating half-life of fewer than 4 hours.[72,74] This longer half life allows for increased accuracy when indicating vitamin D status, as well as 25(OH)D levels are equally affected by all sources of vitamin D (e.g., UV irradiation, dietary intake, and parenteral intake), making it important for assessing the safety and efficacy of vitamin D supplementation.[72,73] As a result, vitamin D sufficiency is defined as serum 25(OH)D levels greater or equal to 75 nmol/L. Levels less than this cut-off are broken into two categories, with levels between 50-74 nmol/L being defined as insufficient and levels <50 nmol/L being defined as deficient.[70]

Concentrations of 1,25$(OH)_2$D should not be used for detecting vitamin D deficiency because the level may be normal or elevated in a person who is vitamin D deficient.[75] When vitamin D levels are low, calcium absorption is impaired and calcium requirements for bone health are not met. This results in increased PTH production to

increase resorption of calcium from the kidneys and stimulates $1,25(OH)_2D$ production as a result of secondary hyperparathyroidism.[75] However, PTH-simulated $1,25(OH)_2D$ levels are still inadequate in maintaining mineral homeostasis. $1,25(OH)_2D$ production is restricted by 25(OH)D substrate availability and therefore, supplementation is still required to improve 25(OH)D and $1,25(OH)_2D$ levels.[76]

2.5 Conclusion

Vitamin D can be administered orally or parentally, resulting in different responses to vitamin D supplementation depending on the route of administration. Oral supplementation will result in a rapid increase in vitamin D levels, which will fall linearly thereafter with high doses or remains at a steady state level with low daily doses[77,78], wherein intramuscular vitamin D administration delays vitamin D bioavailability as there is a slow linear increase in serum 25(OH)D levels, peaking around 6-8 weeks.[79] Overall, it best to assess vitamin D status using levels of 25(OH)D and this will determine whether effective dosing to correct vitamin D deficiency has been undertaken.

3 METHODS

3.1 Brief Study Overview

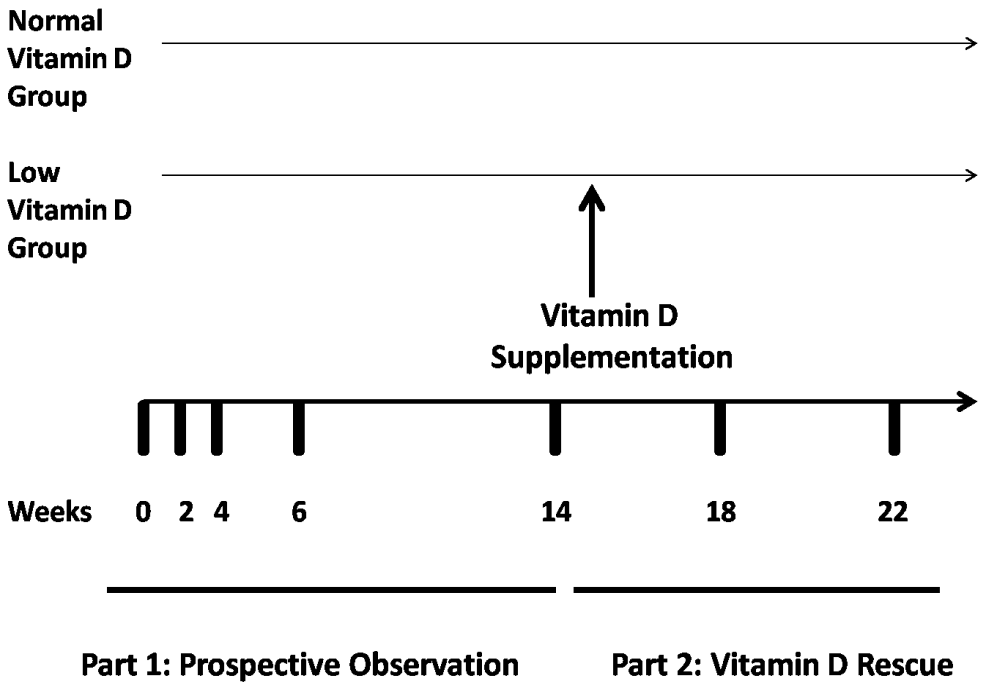


Figure 3-1 Research Design. This was a prospective intervention cohort study of Crohn's disease and ulcerative colitis patients who were starting anti-TNF therapy, infliximab or adalimumab, with no previous exposure to these drugs. Infliximab is administered intravenously at week 0, week 2, week 6, and then every 8 weeks. Adalimumab is administered subcutaneously every 2 weeks. Patients were grouped as having normal vitamin D levels or low vitamin D levels at baseline and then were followed for 22 weeks. This included data collection at week 0, 2, 4, 6, 14, and week 22. Patients in the low vitamin D group were supplemented with vitamin D at week 14 after their infliximab infusion or adalimumab injection.

3.2 Setting and Subject Selection

Eligible subjects were identified by the patient's gastroenterologist from the University of Alberta and Misericordia Hospital. Patients were recruited prospectively as outpatients from the University of Alberta Inflammatory Bowel Disease Clinic Outpatient Clinic and infliximab infusion clinics across Edmonton, Alberta, and as inpatients from the University of Alberta hospital.

3.2.1 Inclusion Criteria

1. A diagnosis of Crohn's disease or ulcerative colitis confirmed by endoscopy and histology
2. Initiating anti-TNF therapy (infliximab or adalimumab)
3. Male or female
4. 18 years of age or older and able to give written consent

3.2.2 Exclusion Criteria

1. Previous exposure to anti-TNF therapy
2. Supplementing with vitamin D > 2000 IU/day orally
3. Received a vitamin D injection < 2 months prior to starting anti-TNF therapy
4. Pregnant and lactating women
5. Patients at increased risk of vitamin D toxicity with treatment
    i. history of cancer
    ii. impaired renal function
    iii. impaired liver function
6. Coeliac disease

3.3 Study Overview

Subjects who met the inclusion and exclusion criteria were invited to participate in this study. The study coordinator discussed with the patients the background and purpose of the study, the study methods, as well as confidentiality and voluntary participation.

Patients were given the opportunity to ask questions and discuss the study. After this discussion, each patient read the information sheet (Appendix A) and signed the informed consent form approved by the University of Alberta Ethics Review Board (Appendix B). Once the consent form was completed, the participants were given a demographics questionnaire (Appendix C), a clinical disease activity questionnaire, either the Harvey Bradshaw Index (HBI) questionnaire for Crohn's disease (Appendix D) or Partial Mayo Score (PM) questionnaire for ulcerative colitis (Appendix E), as well as one quality of life assessment questionnaire (Appendix F) and one depression assessment questionnaire (Appendix G). Baseline disease activity was obtained at the time the patient started the infliximab or adalimumab; however, some patients were on concomitant therapy, such as corticosteroids, at their first dose of anti-TNF therapy, which reduced their clinical disease activity questionnaire scores to totals that were not reflective of their actual disease state at the time their physician decided to start infliximab or adalimumab treatment; therefore, the clinical disease activity score obtained at the time the physician decided to start anti-TNF therapy was also obtained from each patient's chart. The score at the time the decision was made to start anti-TNF therapy was used as the baseline score unless this data was not available.

If the patients were starting infliximab, the infusion nurse drew the blood work immediately before the start of the infusion. If the patients were starting adalimumab, patients were asked to complete the blood work at any laboratory by trained technicians within 1 week prior to their first adalimumab injection. In that some patients were on concomitant therapy, their CRP at this time was not reflective of their disease state. As a result, each patient's baseline CRP level was obtained from the blood work completed at the time the decision to start anti-TNF therapy was made. Subsequently, these levels were then able to be linked with the HBI or PM score obtained at that time. Patients were followed up for 22 weeks, which included the first 5 infliximab infusions (week 0, 2, 6, 14, and 22) or the first 12 adalimumab injections (week 0-12). If patients were dose escalated, wherein the frequency of drug administration was changed,

patients continued to be followed up at week 14 and week 22, regardless of the infusion or injection number. The follow up blood work was completed at week 14 and week 22 immediately before each patient's infliximab infusion and the disease activity, quality of life, and depression assessment questionnaires were completed during these infusions. The follow up blood work, disease activity, quality of life, and depression assessment questionnaires for patients on adalimumab were completed within one week prior to these injections. Patients who were in the low vitamin D group were administered a vitamin D injection within 2 weeks after their week 14 infliximab infusion or adalimumab injection.

This study contained two parts. Part 1 included weeks 0 to 14 and part 2 included weeks 14 to 22, after vitamin D supplementation. A detailed outline of all the data collected for weeks 0, 2, 4, 6, 14, 18, and 22 are as follows:

3.3.1 Part 1 Prospective Observation

At week 0, 2, 4, 6, and 14, disease activity was assessed with:

- Harvey Bradshaw Index (HBI) score for patients with Crohn's disease
- Partial Mayo (PM) clinic score for patients with ulcerative colitis

At week 0 and week 14 the following was measured from the blood samples collected:

- Complete blood count (CBC) labs: Hemoglobin, white blood cell count, platelets; C-reactive protein (CRP); serum 25-hydroxyvitamin D; calcium; albumin; and parathyroid hormone (PTH) levels
- Serum cytokine levels (IL-1beta, IL-2, IL-6, IL-8, IL-10, IL-12, GM-CSF, TNF-alpha, INF-gamma)

At week 0 and week 14 the following questionnaires were administered to assess quality of life and depression:

- The Short Inflammatory Bowel Disease Questionnaire (SIBDQ) to assess quality of life
- The Beck Depression Inventory-II (BDI-II) questionnaire to assess depression

3.3.2 Part 2 Vitamin D Rescue

Part 2 of the study was initiated at week 14. Patients in the low vitamin D group were given a single intramuscular vitamin D injection within 2 weeks after their week 14 infliximab infusion or after their week 14-adalimumab injection according to the protocol in Table 3.1. This was completed by a nurse in the IBD clinic, by the patient's pharmacist, or by the patient's family doctor. The normal vitamin D group did not receive supplemental vitamin D. Patients were then followed prospectively for an additional 8 weeks.

At week 18 and 22 disease activity was assessed with:

- Harvey Bradshaw Index (HBI) score for patients with Crohn's disease
- Partial Mayo (PM) clinic score for patients with ulcerative colitis

At week 22 the following was measured from the blood samples collected:

- CBC labs: Hemoglobin, white blood cell count, platelets; C-reactive protein (CRP); serum 25-hydroxyvitamin D; calcium; albumin; and parathyroid hormone (PTH) levels
- Serum cytokine levels (IL-1beta, IL-2, IL-6, IL-8, IL-10, IL-12, GM-CSF, TNF-alpha, INF-gamma)

At week 22 following questionnaires were administered to assess quality of life and depression:

- The Short Inflammatory Bowel Disease Questionnaire (SIBDQ) to assess quality of life
- The Beck Depression Inventory- II (BDI-II) questionnaire to assess depression

An overview of the study activity schedule is outlined in Table 3.2.

Table 3-1 Replacement doses of vitamin D, based on serum 25-OH vitamin D levels

Serum 25-OH Vitamin D Level	Cholecalciferol (in sesame oil)
Less than 50 nmol/L	500, 000 IU IM x 1
Less than 75 nmol/L and greater than or equal to 50 nmol/L	250, 000 IU IM x 1

This protocol (Table 3.1) was developed by Dr. R. Fedorak (University of Alberta, Division of Gastroenterology) and Dr. K. Siminoski (University of Alberta, Division of Endocrinology) for the treatment of vitamin D deficiency in IBD patients. This protocol is implemented in the clinical practice of IBD physicians at the University of Alberta and therefore to continue with clinical practice protocols, intramuscular (IM) injections of vitamin D were used in this study to supplement the low vitamin D group. Additionally, a literature review was conducted to assess the efficacy of oral vitamin D versus IM vitamin D in IBD patients. There are no clinical trials comparing these two routes of administration in IBD patients, and in that some IBD patients may be prone to intestinal malabsorption of oral vitamin D[49], intramuscular supplementation has been chosen as the route of administration for this study.

Table 3-2 Study Activity Schedule

	Prospective Observation					*Vitamin D Rescue*	
	Week 0	**Week 2**	**Week 4**	**Week 6**	**Week 14**	**Week 18**	**Week 22**
History	X						
CBC, CRP	X				X		X
Vitamin D	X				X		X
HBI	X	X	X	X	X	X	X
PM	X	X	X	X	X	X	X
Cytokines	X				X		X
Other labs	X				X		X
Vitamin D injection					X		
SIBDQ	X				X		X
BDI-II	X				X		X

3.4 Experimental variables

3.4.1 Independent Variables

The independent variable was the serum 25-hydroxyvitamin D (25(OH)D) level; this determined the patient's vitamin D status. Patients in the normal vitamin D group had a 25(OH)D level ≥ 75 nmol/L and patients in the low vitamin D group had a 25(OH)D level < 75 nmol/L. The literature has defined 2 cut offs to stratify vitamin D status into 3 groups. Vitamin D sufficiency is defined as a 25(OH)D level ≥ 75 nmol, with insufficiency defined as a level <75 and ≥ 50 nmol/L and deficiency as a level ≤ 50 nmol/L.[70] With an expected small sample size, stratifying the patients with low vitamin D into two categories would result in a very small sample size in each group; as a result, for this study, groups were defined as having normal vitamin D levels or low vitamin D levels using the cut-off value of 75 nmol/L.

3.4.2 Dependent Variables

3.4.2.1 Measurements of disease activity

Clinical disease activity questionnaires, the Harvey Bradshaw Index (HBI) (Appendix D) for Crohn's disease and Partial Mayo Score (PM) (Appendix E) for ulcerative colitis, were collected at baseline (week 0), week 2, 4, 6, 14 and 22 to monitor changes in disease activity during anti-TNF therapy in the low and normal vitamin D groups and after vitamin D treatment in the low vitamin D group. The Crohn's Disease Activity Index (CDAI) is commonly used in clinical trials to assess disease activity in patients with Crohn's disease; however, its calculation is complex.[80] The HBI was developed as a simplified version of the Crohn's disease activity index (CDAI), assessing general well-being, abdominal pain, stool frequency, and additional manifestations; it has a 93% correlation with the CDAI.[81] Therefore, the HBI questionnaire was chosen to clinically assess Crohn's disease activity for this study. The 9-point Partial Mayo Score (PM) is based on the Mayo Score without the endoscopy sub-score. Therefore, the PM questionnaire was chosen for this study, assessing stool frequency and rectal bleeding; it is a non-invasive tool for monitoring ulcerative colitis activity and is effective in that it has good sensitivity and specificity in identifying patients in clinical remission or with clinical improvement.[82] The scores of these questionnaires can fall into 4 groups, which define the disease severity of the patient. A patient may be in remission or have mild, moderate, or severe disease activity. These disease activity scores were used to determine clinical response and clinical remission.

It should be noted that these questionnaires do not accurately measure disease activity in Crohn's disease patients with fistulizing disease or stricturing disease. In these cases, stool frequency may be low or high, and in that the HBI score is driven by stool frequency, these patients may not have a score that would represent their actual disease state. Therefore, these patients were excluded from the clinical response and

clinical remission sections of the results. However, these patients were still included in the other sections that assessed disease activity (CRP and cytokines).

3.4.2.1.1 Clinical Definitions

1. ***Clinical response:*** achievement of a HBI score <5 or a decrease in the HBI score by ≥3 points from baseline[80] for Crohn's disease, or achievement of a PM score of 0 or 1, or a decrease in the PM score by ≥3, with a decrease in the rectal bleeding sub-score of ≥1 for ulcerative colitis or absolute rectal bleeding score of 0 or 1. [82]
2. ***Clinical remission:*** achievement of a HBI score <5 for Crohn's disease[80] or achievement of a PM score of 0 or 1 for ulcerative colitis.[82]
3. ***Loss of Response***: Patients who underwent an IBD-related surgery, terminated anti-TNF treatment, or were dose escalated were not considered to have a clinical response from the time of the event onward, regardless of his/her HBI or PM score.
4. ***Clinically significant response***: an increase of ≥ 20% in the proportion of patients who achieve a clinical response compared to baseline. Randomized clinical trials examining efficacy of infliximab and adalimumab in moderate to severe IBD have reported 20% difference in response rates to be clinically significant.[83]
5. ***Dose escalation***: If a patient loses clinical response, i.e., patients were developing disease symptoms, optimization of anti-TNF treatment is the primary intention. For infliximab, the interval of drug administration can be decreased from 8 weeks to 4 weeks or the dose per kg can be increased from 5mg/kg to 10 mg/kg. For adalimumab, the maintenance frequency of drug administration can be increased from every 2 weeks to every week, still at 40 mg; dose is not changed.[84]
6. ***IBD-related surgery***: Patients would be defined as a non-responder to infliximab or adalimumab if after the induction phase of the drug patients were sent to surgery to remove part of their intestine because of unresolved inflammation or severe stricturing (including small bowel resection, ileocecal resection, colectomy, and segmental colon resection) (personal communications, Dr. Haili Wang).

3.4.2.2 Serological Disease Activity Markers

3.4.2.2.1 C reactive protein

C reactive protein (CRP) was measured as a surrogate marker of systemic inflammation. It was measured at baseline, week 14, and week 22 to assists in the interpretation of disease activity. CRP response at week 14 or week 22 was defined as a decrease in the patient's baseline CRP level by ≥ 50%. CRP remission was defined as achievement of a CRP levels < 8 mg/L at week 14 or week 22. There are, however, patients who do not produce CRP due to genetic variability[85]; as a result, these patients were excluded from the CRP analysis.

3.4.2.2.2 Experimental Cytokines

IL-1beta, IL-2, IL-6, IL-8, IL-10, IL-12, GM-CSF, TNF-alpha, and INF-gamma were measured to assist in the interpretation of disease activity and to understand the influence of vitamin D on immune responses. This panel was used because these cytokines are related to the pathogenesis and course of IBD as well as are regulated by vitamin D.

3.4.2.2.3 Other

Calcium and parathyroid hormone were measured at baseline, week 14 and week 22 to ensure normality of the vitamin D metabolism pathway[50]. Complete blood work (CBC) and albumin was measured at baseline, week 14, and week 22 as these are markers of wellness.[86]

3.4.2.3 Management of quality of life and depression

3.4.2.3.1 Short inflammatory bowel disease questionnaires

The Short Inflammatory Bowel Disease Questionnaire (SIBDQ) by Irvine et al.[87] was chosen for this study as it is an IBD specific questionnaire developed from a cohort of Crohn's disease and ulcerative colitis patients to assess patients' response to interventions in terms of changes in health related quality of life (Appendix F). The SIBDQ is the short version of the inflammatory bowel disease questionnaire (IBDQ),

which is a physician-administered disease-specific health-related quality of life (HRQOL) questionnaire that defines changes in health status in IBD.[87,88] They both provide a subjective assessment of quality of life; however, the SIBDQ has fewer questions (10 vs. 32) and is self-administered; it has been proven to be valid and reliable.[87] There are 10 multiple-choice questions, wherein each question contains 7 statements. Depending on the answer, each question receives a score from 1-7. Total scores were reported with a 7-point scale (1=poor HRQoL, 7=optimum HRQoL).

3.4.2.3.2 Beck depression inventory – second edition (BDI-II)

The BDI-II was created by Dr. Aaron Beck[89], and the second version of this questionnaire was published in 1966 (Appendix G). It was chosen for this study as it is a commercially available, patient administered questionnaire, used in adults and adolescents who are 13 years and older to measure severity of depression. There are 21 multiple-choice questions, wherein each question contains 4 statements that range in severity from 0-3. The overall severity of depression is based on the total score, wherein higher scores indicate more severe depression. The range of possible scores for the BDI-II is 0-62, and severity of depression is categorized into 4 groups: minimal (0-13), mild (14-19), moderate (20-28), and severe (29-63)[89]. Furthermore, Thomas et al.[90] developed a model wherein the BDI-II questions were divided into cognitive symptoms (i.e., sadness, loss of pleasure, guilty feelings) and somatic symptoms (i.e., loss of energy, irritability, and fatigue) of depression, and this model was chosen for this study. Questions 1-14 were summed to determine the cognitive score and questions 15-21 were summed to determine the somatic score.

3.5 Overview of Specimen Handling and Processing

Blood draws for haematology, chemistry, endocrinology, and biochemistry were performed by infliximab infusion nurses or by trained technicians at a local pathology laboratory as part of standard patient care. Edmonton zone lab services analyze samples from multiple locations in Edmonton, and analyzed our samples. Samples were stored

for vitamin D analysis in a -20 degree Celsius freezer. Vitamin D levels were measured using Ultra Performance Liquid Chromatography to accurately separate the forms of vitamin D (25-hydroxyvitamin D2 and 25-hydroxyvitamin D3), with a coefficient of variation of 5% (personal communications, Keith Steinbach). In that the coefficient of variation is very small, only one vitamin D level was measured for each patient at each time point. Although using one measurement of vitamin D was a limitation of the study, this method is used regularly in clinical practice among all patients populations, and therefore, was a 'real life' measurement of vitamin D status.

The infliximab infusion nurses drew blood for cytokine analysis just prior to the start of each patient's infliximab infusion. These specimens were collected in a gold top 5 ml tube. Samples were spun and aliquoted within 2 hours of collection. Samples were stored in a -70 degree Celsius freezer for up to 18 months to a few weeks before analysis; cytokines are stable for up to 2 years when frozen.[91] The Meso Scale Discovery Multi-Spot Assay System, Human Pro-Inflammatory 9-Plex Ultra-Sensitive Kit (K15007C-1) was used to measure IL-2, IL-8, IL-12p70, IL-1beta, GM-CSF, IFN-gamma, IL-6, IL-10, TNF-alpha cytokine levels. Samples were thawed on ice and the assay protocol was performed according to instructions over two days to increase the sensitivity of the assay.

3.6 Medication and Supplementation

All medications were permitted as clinically indicated. Corticosteroids increase degradation of 25-OH vitamin D[92]; however, this is a standard medication used in treatment of IBD patients who are not in remission[15] and therefore was permitted. An oral dose of 2000 IU/day is the upper limit of recommended daily vitamin D intake,[93] therefore, a dose greater than 2000 IU per day was not permitted. Dietary vitamin D is not a concern for this study as only a small amount of vitamin D comes from the diet.[93]

3.7 Data Management

All study subjects were assigned a study number that appeared on all data collection instruments, documents, and files. Personal information needed for tacking and informed consent forms were stored separately from the other data with only limited team members having access. This was to ensure that the privacy and confidentiality of the participants in the study were protected.

3.8 Statistical Analysis

Dichotomous end points (i.e., response and remission) were compared using Chi-square tests or Fisher's exact tests, where appropriate. The risk difference and relative risk were reported with their respective 95% confidence intervals (CI). Continuous variables of independent samples were compared using Mann-Whitney U Tests and continuous variables of paired-samples were compared using the Wilcoxon signed rank test. Medians were reported with its respective interquartile range (IQR).

For subgroup analysis of these outcomes, patients were stratified by disease severity, assessed by the HBI or PM score before initiating anti-TNF therapy. Patients were grouped as having severe disease, defined by a HBI score > 7 or PM score > 4 or as having non-severe disease, defined by a HBI score < 8 or a PM score < 5.

In response to a missing vitamin D value at week 14, the last observation was carried forward (LOCF). All other variables were determined missing if they were not obtained at the appropriate time point, and the patient was excluded from that type of analysis. All statistical tests were two-sided and performed at the 0.05 level of significance. Statistical analyses were conducted using IBM SPSS Statistics version 21.

3.9 Controlling for Bias

Bias was first controlled for during patient recruitment. Patients were not included in the study if they had coeliac disease, cancer, or were at risk of vitamin D toxicity, as

these diseases interfere with vitamin D absorption and metabolism. Furthermore, patients were excluded if they had been previously exposed to anti-TNF therapy, as this exposure decreases their ability to respond to a different anti-TNF therapy.[94] Bias was also controlled for during statistical analysis. Baseline characteristics were collected using the demographics form to determine if there were differences in these variables between the groups. Moreover, to control for seasonal variation in vitamin D, patients were recruited all year round.

3.10 Ethical Considerations

This study was approved by the University of Alberta Health Research Ethics Board (Pro00031844).

4 RESULTS

4.1 STUDY SUBJECT FLOW CHART (FIGURE 4-1)

Patients were recruited from October 2012 to January 2014. Written consent was obtained from 62 subjects who met the inclusion criteria. Patients were categorized into either the normal vitamin D or low vitamin D group, defined by their baseline vitamin D level that was measured before they started anti-TNF therapy. There were 8 patients who were withdrawn from the study after recruitment because of the following reasons:

1. Patient received a vitamin D injection before week 14 of the study (n=2, low vitamin D group)
2. Patient was re-induced on infliximab or had multiple infusions when started in hospital (n=3; 2 low vitamin D group, 1 normal vitamin D group)
3. Patient stopped the drug after developing an infection (n=1, normal vitamin D group)
4. Patient was diagnosed with cancer while in the study (n=2, low vitamin D group)

In that there were two parts to this study, patients were categorized into two groups: patients who completed up to week 14 of the study (completed part 1) and those who completed up to week 22 of the study (completed part 1 and part 2). There were 54 patients who completed up to week 14 and 43 patients who completed up to week 22. Of these 43 patients, 5 were excluded for the following reasons:

1. Patient requested withdrawal after week 14 of study (n=1, low vitamin D group)
2. Patient did not receive vitamin D injection by week 22 (n=2, low vitamin D group)
3. Patient's infliximab dosing changed to 6 weeks after week 18 (n=1, normal vitamin D group)

4. Patient stopped infliximab therapy due to non-compliance (n=1, normal vitamin D group)

As a result, there were 38 patients who completed up to week 22 of the study. Figure 4-1 describes the overall study subject flow.

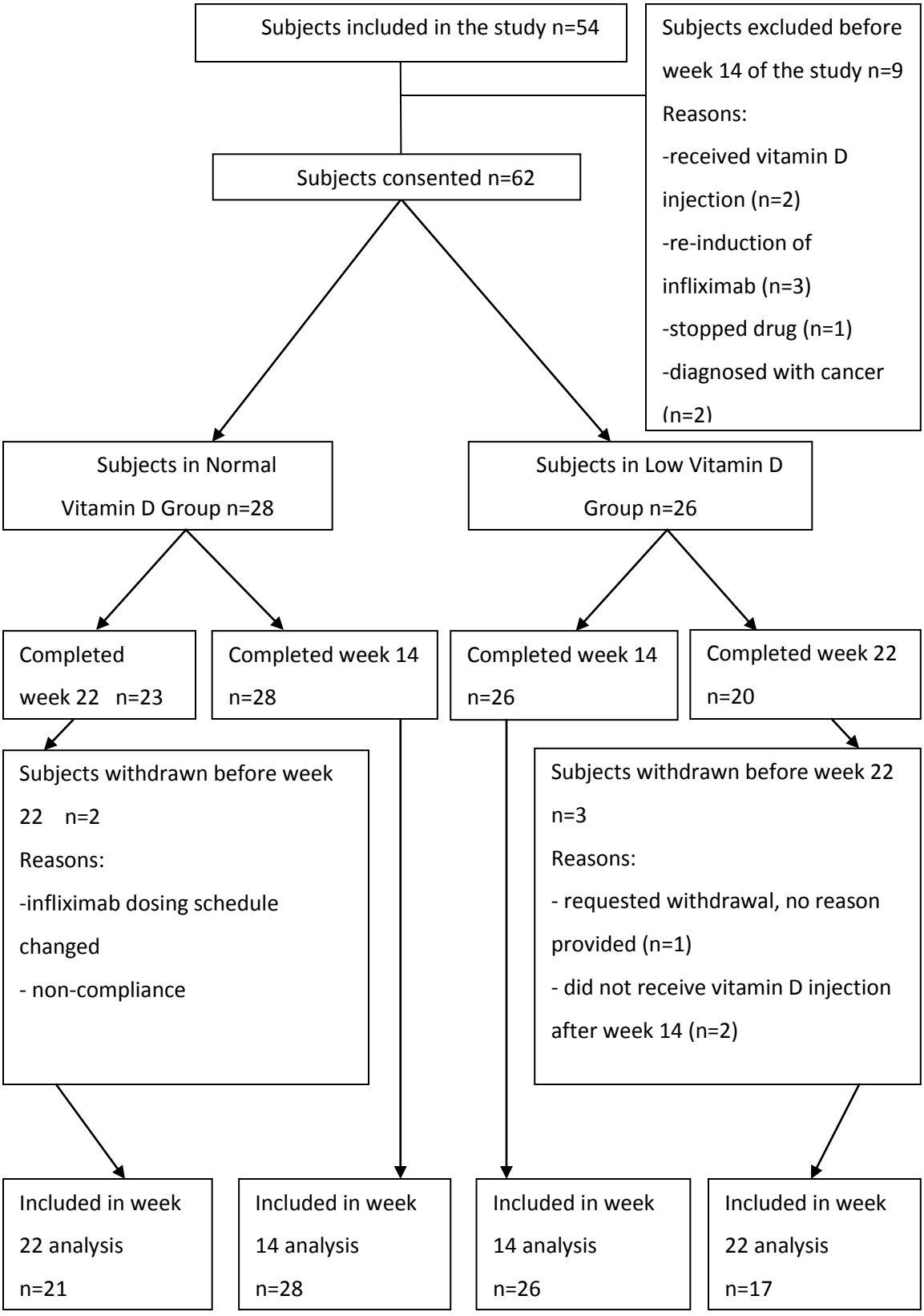


Figure 4-1 Study flowchart from recruitment to analysis

4.2 BASELINE CHARACTERISTICS

Baseline characteristics of patients who completed up to week 14, stratified by vitamin D status, are similar as presented in Table 4.1. Baseline characteristics of patients who completed up to week 22, stratified by vitamin D status, are presented in Table 4.3. Patients with low vitamin D levels were younger, and all other demographics were similar. Baseline labs of patients who completed up to week 14 are presented in Table 4.2 and baseline labs of patients who completed up to week 22 are presented in Table 4.4. All patients prescribed infliximab were initiated on a dose of 5 mg/kg and completed the induction doses at week 0, 2, and 6. All patients prescribed adalimumab were initiated on a dose of 160 mg at week 0, then 80 mg at week 2, and then 40 mg at week 4. By week 14, vitamin D levels of patients in the low vitamin D group continued to be lower than 75 nmol/L and vitamin D levels of patients in the normal vitamin D group continued to be above 75 nmol/L. By week 22, all patients had a vitamin D levels above 75 nmol/L.

Table 4-1 Demographics of IBD patients who completed up to week 14, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=28)	Low vitamin D Group (n=26)	P-value
Age (years) Median (IQR)	40.5 (30.0-55.5)	34.0 (22.0-39.0)	0.070
Male %, n	50%, 14	69%, 18	0.150
Disease Duration (years) Median (IQR)	5.0 (0.0-14.0)	10.0 (2.0 – 18.0)	0.440
Crohn's Disease %, n	79%, 22	81%, 21	0.840
Disease Location %, n			
Colonic	21%, 6	31%, 8	0.950
Ileal	39%, 11	35%,9	
Ileocolonic	18%, 5	15%, 4	
Pancolitis	11%, 3	7%, 2	
Left-sided colitis	11%, 3	12%, 3	
Disease Behavior %, n			
Inflammatory	79%, 22	84%, 22	0.380
Penetrating	14%, 4	4%, 1	
Stricturing	7%, 2	12%, 3	
Infliximab %, n	93%, 26	89%, 23	0.580
Smoking Status %, n			
Current	32%, 9	23%, 6	0.560
Nonsmoker	39%, 11	46%, 12	
Former Smoker	29%, 8	31%, 8	
History of Surgery %, n	25%, 7	19%, 5	0.610
Sulfasalazine/mesalamine %, n	25%, 7	19%, 5	0.610
Immunosuppressants %, n	75%, 21	73%, 19	0.870
Corticosteroids %, n	50%, 14	62%, 16	0.400

Table 4-2 Baseline labs of patients who completed up to week 14, stratified by vitamin D status

Labs	Normal Vitamin D Group (N=28)	Low vitamin D Group (N=26)	P value
Vitamin D (nmol/L) Median (IQR)	97.5 (84.0-105.0)	52.5 (46.0-65.0)	<0.001
Calcium (mmol/L) Median (IQR) Ref. Range 2.10-2.60	2.32 (2.27-2.42)	2.26 (2.21-2.32)	0.032
Albumin (g/L) Median (IQR) Ref. Range: 35-50	42.0 (38.0-43.0)	39.0 (35.0-41.0)	0.017
PTH (pmol/L) Median (IQR) Ref. Range: 1.4-6.8	3.8 (2.8-4.5)	3.3 (2.9-4.6)	0.660
Hemoglobin (g/L) Median (IQR) Ref. Range: 120-160	137.5 (123.5-148.5)	128.0 (110.0-141.0)	0.077
White Blood Cells (10^9/L) Median (IQR) Ref. Range: 4.0-11.0	7.3 (5.6-10.3)	7.4 (5.8-9.3)	0.680
Platelets (10^9/L) Median (IQR) Ref. Range: 140-450	258.0 (215.5-315.0)	313.0 (256.0-384.0)	0.085

Table 4-3 Demographics of patients who completed up to week 22, stratified by vitamin D

Characteristics	Normal Vitamin D Group (n=21)	Low vitamin D Group (n=17)	P-value
Age (years) Median (IQR)	46.0 (31.0-60.0)	27.0 (22.0-36.0)	0.004
Male %, n	43%, 9	71%, 12	0.087
Disease Duration (years) Median (IQR)	5 .0 (0.0-14.0)	10.0 (2.0-15.0)	0.580
Crohn's Disease %, n	76%, 16	82%, 14	0.640
Disease Location %, n			
Colonic	19%, 4	23%, 4	0.830
Ileal	33%, 7	47%, 8	
Ileocolonic	24%, 5	12%, 2	
Pancolitis	10%, 2	6%, 1	
Left-sided colitis	14%, 3	12%, 2	
Disease Behavior %, n			
Inflammatory	86%, 18	82%, 14	0.690
Penetrating	10%, 2	6%, 1	
Stricturing	4%, 1	12%, 2	
Infliximab %, n	91%, 19	88%, 15	0.820
Smoking Status %, n			
Current	24%, 5	18%, 3	0.620
Nonsmoker	43%, 9	59%, 10	
Former Smoker	33%, 7	23%, 4	
History of Surgery %, n	24%, 5	24%, 4	0.950
Sulfasalizine/mesalamine %, n	24%, 5	18%, 3	0.640
Azathioprine %, n	76%, 16	88%, 15	0.340
Corticosteroids %, n	43%, 9	65%, 11	0.180

Table 4-4 Baseline labs of patients who completed up to week 22, stratified by vitamin D status

Labs	Normal Vitamin D Group (N=21)	Low vitamin D Group (N=17)	P value
Vitamin D (nmol/L) Median (IQR)	98.0 (87.0-104.0)	54.0 (46.0-66.0)	<0.001
Calcium (mmol/L) Median (IQR) Ref. Range 2.10-2.60	2.29 (2.27-2.39)	2.23 (2.18-2.31)	0.170
Albumin (g/L) Median (IQR) Ref. Range: 35-50	40.0 (37.0-43.0)	38.5 (35.0-41.0)	0.096
PTH (pmol/L) Median (IQR) Ref. Range: 1.4-6.8	4.0 (2.9-5.0)	3.0 (2.1-3.7)	0.070
Hemoglobin (g/L) Median (IQR) Ref. Range: 120-160	133.0 (122.0-146.0)	126.0 (117.0-134.0)	0.160
White Blood Cells (10^9/L) Median (IQR) Ref. Range: 4.0-11.0	7.2 (5.2-8.7)	6.8 (5.6-10.3)	0.930
Platelets (10^9/L) Median (IQR) Ref. Range: 140-450	263.0 (220.0-325.0)	320.0 (260.0-406.0)	0.095

4.3 PRIMARY ENDPOINT: EFFICACY AT WEEK 14

4.3.1 Patient Population

There were 54 patients who completed up to week 14 of the study. Of these patients, there were 10 patients who had fistulizing disease or stricturing disease. In that the HBI scores recorded from these patients are not reflective of their disease state, these patients were excluded from the clinical response and remission analysis as well as from the analysis of the raw disease activity scores. Patients who had multiple resections or an ostomy were included in this analysis. The number of bowel movements these patients were having was not different from the other patients, and clinical response was still able to be determined. Therefore, the total number of IBD patients analyzed was 44. There were 33 Crohn's disease patients, with non-stricturing, non-penetrating disease behavior, and 11 ulcerative colitis patients, who started anti-TNF therapy due to disease inflammation; patients with concomitant perianal disease were included. Baseline demographics were similar between the two groups. The demographics of these patients are presented in Table 4.5 and the baseline blood work is presented in Table 4.6.

Table 4-5 Efficacy at week 14: Demographics of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=21)	Low vitamin D Group (n=23)	P-value
Age (years) Median (IQR)	43.5 (30.0-57.0)	36.0 (22.0-49.0)	0.200
Male %, n	48%, 10	65%, 15	0.240
Disease Duration (years) Median (IQR)	5.0 (0.0 – 17.0)	9.0 (2.0-18.0)	0.440
Crohn's Disease %, n	71%, 15	78%, 18	0.600
Disease Location %, n Colonic Ileal Ileocolonic Pancolitis Left-sided colitis	 15%,3 33%,7 24%,5 14%,3 14%,3	 30%,7 39%,9 9%,2 9%,2 13%,3	0.520
Disease Behavior %, n Inflammatory Penetrating Stricturing	100%, 21	100%, 23	1.00
Infliximab %, n	91%, 19	87%, 20	0.710
Smoking Status %, n Current Nonsmoker Former Smoker	 19%, 4 48%, 10 33%, 7	 17%, 4 44%, 10 39%, 9	0.920
History of Surgery %, n	29%, 6	17%, 4	0.480
Sulfasalazine/mesalamine %, n	29%, 6	26%, 6	0.850
Immunosuppressants %, n	71%, 15	70%, 16	0.890
Corticosteroids %, n	43%, 9	61%, 14	0.230

Table 4-6 Efficacy at week 14: Baseline labs of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Labs	Normal Vitamin D Group (N=21)	Low vitamin D Group (N=23)	P value
Vitamin D (nmol/L) Median (IQR)	92.0 (81.0-101.0)	52.0 (44.0-66.0)	<0.001
Calcium (mmol/L) Median (IQR) Ref. Range 2.10-2.60	2.29 (2.26-2.41)	2.25 (2.19-2.30)	0.039
Albumin (g/L) Median (IQR) Ref. Range: 35-50	41.0 (37.0-43.0)	38.5 (35.0-41.0)	0.046
PTH (pmol/L) Median (IQR) Ref. Range: 1.4-6.8	3.9 (3.1-4.3)	3.3 (3.1 – 4.7)	0.680
Hemoglobin (g/L) Median (IQR) Ref. Range: 120-160	135.0 (122.0-148.0)	127.0 (109.0–141.0)	0.140
White Blood Cells (10^9/L) Median (IQR) Ref. Range: 4.0-11.0	7.1 (5.2 – 8.5)	7.9 (6.0 – 10.3)	0.340
Platelets (10^9/L) Median (IQR) Ref. Range: 140-450	271.5 (218.0-325.0)	293.5 (252.0-384.0)	0.240

4.3.2 Clinical Response At Week 14

4.3.2.1 Clinical response in patients with IBD (including CD and UC)

The proportion of IBD patients in the normal vitamin D group who responded at week 14 was 67% (14/21). Similarly, 65% (15/23) of patients in the low vitamin D group responded at week 14 (p=0.92) (Figure 4-2 A). Therefore, the relative risk (RR) of achieving a clinical response at week 14 if a patient had normal vitamin D levels before initiating anti-TNF therapy was 1.0 (95% CI: 0.67 -1.6).

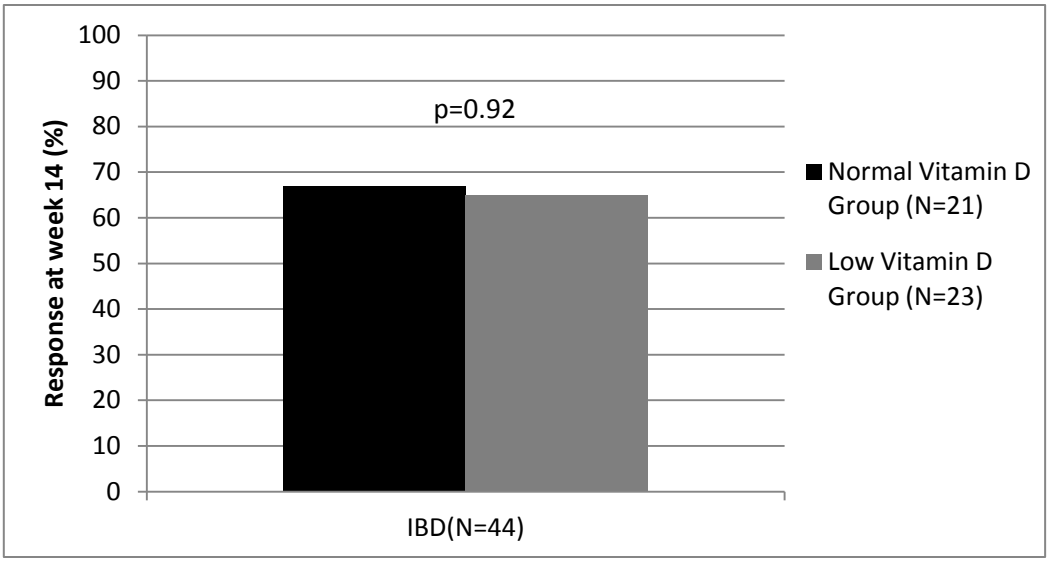
4.3.2.2 Clinical response in IBD patients with severe and non-severe disease

Patients were stratified by disease severity based on their baseline disease activity questionnaire score. There were 31 patients with severe disease, defined by a baseline HBI score of > 7 or a baseline PM score > 5, and 13 patients with non-severe disease.

Among patients with severe disease, the proportion of patients who clinically responded at week 14 in the low vitamin D group was 26% (95% CI: -6.0% - 58%) higher than the proportion of patients in the normal vitamin D group (79% (11/14) vs. 53% (9/17), p=0.14), as presented in Figure 4-2B. As a result, IBD patients with severe disease and low vitamin D levels before initiating anti-TNF therapy were 1.5 (95% CI: 0.88-2.5) times more likely to respond at week 14 than patients with severe disease and normal vitamin D levels.

In contrast, among patients with non-severe disease, the proportion of patients who clinically responded at week 14 in the normal vitamin D group was 56% (95% CI 23%-88%) higher than the proportion of patients who in the low vitamin D group (100% (4/4) vs. 44% (4/9), p=0.11; RR: 2.3, 95% CI: 1.1 - 4.7), as presented in Figure 4-2B. Therefore, IBD patients with non-severe disease and normal vitamin D levels before initiating anti-TNF therapy were 2.3 times more likely to respond clinically at week 14 than patients with non-severe disease and low vitamin D levels.

A.



B.

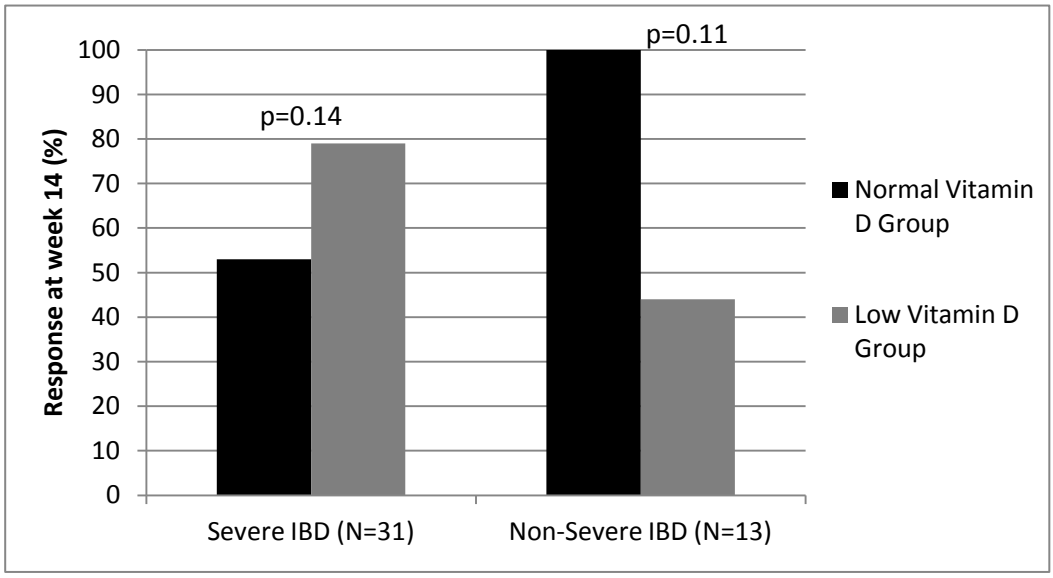


Figure 4-2 Clinical Response at week 14. A. The proportion of patients with IBD on anti-TNF therapy, who responded at week 14, stratified by vitamin D status**. B.** The proportion of IBD patients on anti-TNF therapy who responded at week 14, stratified by vitamin D status, in the severe-disease group and non-severe disease group. IBD patients with low vitamin D before initiation of anti-TNF therapy have a stronger clinical response to therapy, if they have severe disease.

4.3.2.3 Clinical response in patients with Crohn's disease and ulcerative colitis

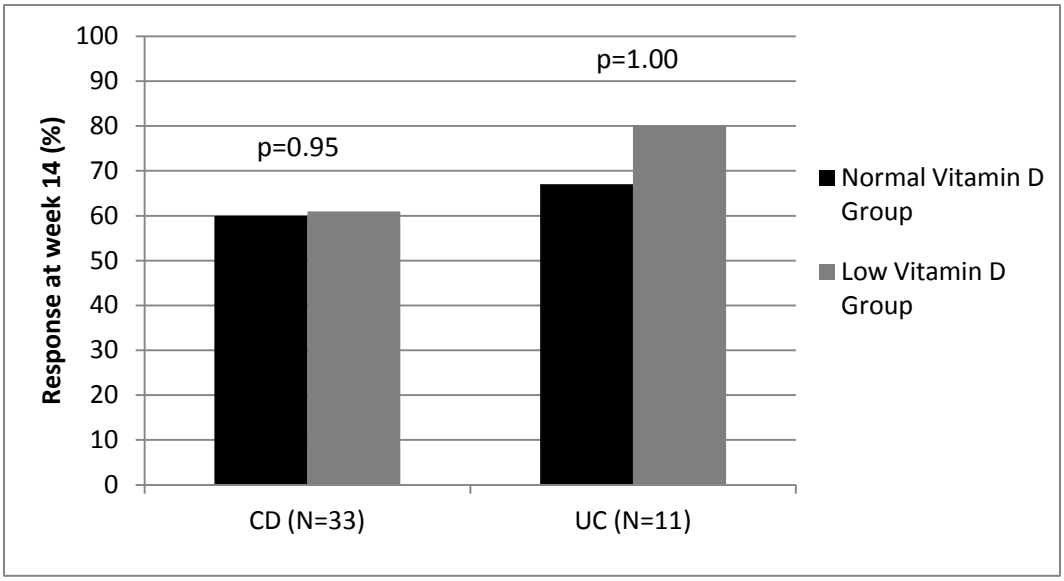
There were 33 patients with a diagnosis of Crohn's disease, including 15 patients in the normal vitamin D group and 18 patients in the low vitamin D group, and 11 patients with a diagnosis of ulcerative colitis, including 6 patients in the normal vitamin D group and 5 patients in the low vitamin D group.

The proportion of patients with Crohn's disease who responded, as per the minimum 3-point drop in their HBI score from baseline at week 14, was similar in the normal and low vitamin D groups (60% (9/15) vs. 61% (11/18), p=0.95; RR: 0.98, 95% CI: 0.56-1.7). Furthermore, the proportion of patients with ulcerative colitis who responded at week 14 in the normal vitamin D group was 67% (4/6) and 80% (4/5) in the low vitamin D group (p=1.00), with a relative risk of 0.83 (95% CI: 0.41-1.7), as presented in Figure 4-3 A.

4.3.2.4 Clinical response in patients with severe and non-severe CD and UC

After stratifying by disease severity, the response trends in the Crohn's disease group were similar to those demonstrated in the IBD patients. As a result, among patients with severe Crohn's disease, there proportion of patients who responded at week 14 in the low vitamin D group was 32% (-7.0% - 72%) higher than the proportion of patients in the normal vitamin D group (78% (7/9) vs. 46% (5/11), p=0.14; RR of 1.7 (95% CI: 0.82-3.6). Furthermore, 100% (4/4) of patients in the normal vitamin D group responded at week 14 compared to 44% (4/9) of patients in the low vitamin D group (p=0.11), among patients with non-severe Crohn's disease, with a relative risk of 2.3 (95% CI: 1.1-4.7) (Figure 4-3B). All the ulcerative colitis patients had severe disease at baseline.

A.



B.

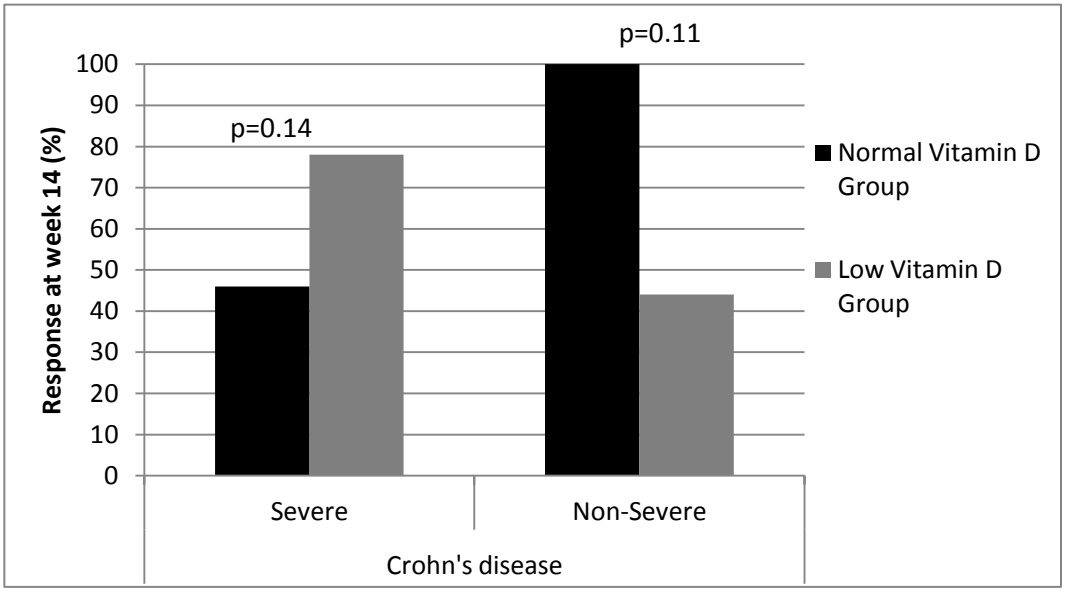


Figure 4-3 Clinical response at week 14 in CD and UC. A. The proportion of patients with Crohn's disease and ulcerative colitis on anti-TNF therapy, who clinically responded at week 14, stratified by vitamin D status. **B**. The proportion of Crohn's disease patients on anti-TNF therapy who responded at week 14, stratified by vitamin D status, in the severe-disease and non-severe disease groups. Crohn's disease and ulcerative colitis patients with low vitamin D before initiation of anti-TNF therapy have a stronger clinical response to therapy, if they have severe disease.

4.3.3 Clinical Disease Activity Scores At Week 14

4.3.3.1 Harvey Bradshaw Index scores for Crohn's disease

Disease activity was assessed in Crohn's disease patients by the Harvey Bradshaw Index (HBI) questionnaire at weeks 0, 2, 4, 6, and week 14. The trend in scores over 14 weeks is presented in Figure 4-4. One patient with Crohn's disease underwent surgery before week 14 and was excluded from this HBI analysis.

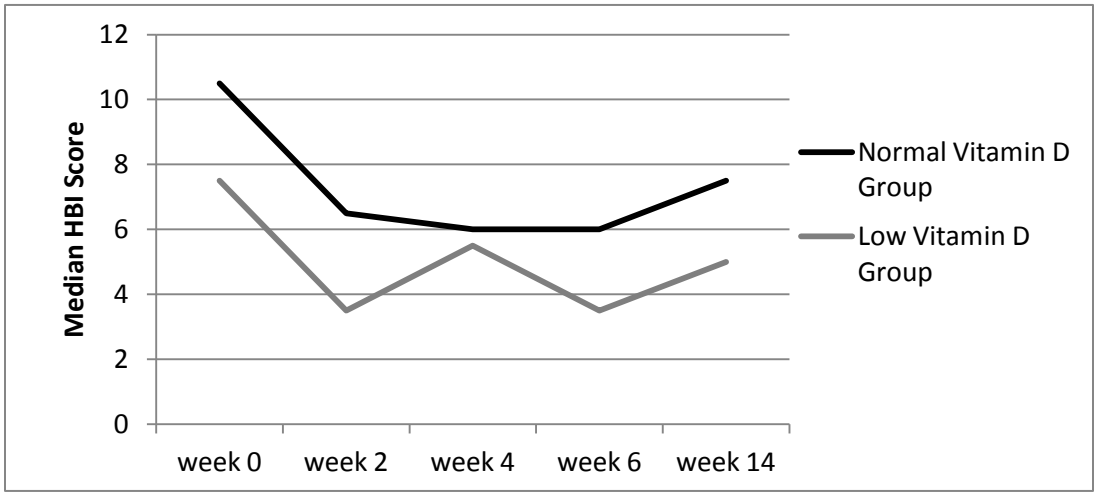


Figure 4-4 HBI scores over 14 weeks of anti-TNF therapy. Patients in both vitamin D groups had the greatest response at week 2 and maintained this response up to week 14.

4.3.3.1.1 Baseline HBI scores

At baseline, the median HBI scores in each group (Figure 4-4) represented moderate disease, which ranges from scores of 8-16. The normal vitamin D group had a median score of 10.5 (IQR: 7.0-15.0) and the low vitamin D group had a median 7.5 (IQR: 6.0-10.0), p=0.071.

4.3.3.1.2 Week 14 HBI scores

By week 14, there was a decrease in median HBI scores in each group, reflecting improvement in both groups (Figure 4-4). The normal vitamin D group, however, still

had a median HBI score in the moderate disease range (7.5, IQR: 4.0-9.5) and the low vitamin D group had a median HBI score in the mild disease range (5.0, IQR: 3.8 – 6.3), (p=0.099).

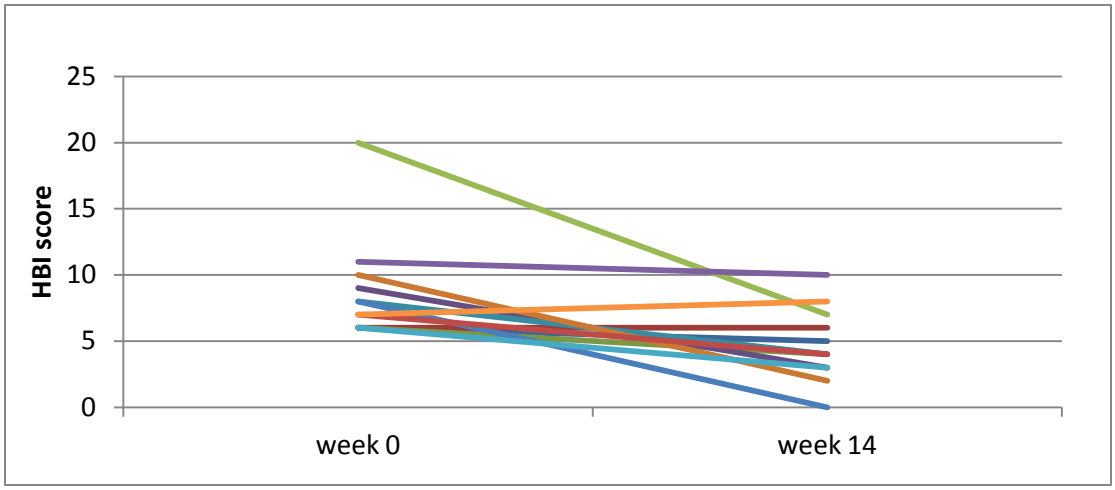
4.3.3.1.3 Within group-differences between week 14 and week 0 HBI scores

There was a drastic difference between the median HBI scores at week 14 and week 0 within the group of Crohn's disease patients who had normal vitamin D levels (10.5, IQR: 7.0 -15.0 vs. 7.5, IQR: 4.0- 9.5, p=0.010), as well as within the group of Crohn's disease patients who had low vitamin D levels (7.5 IQR: 6.0-10.0 vs. 5.0, IQR: 3.8-6.3, p=0.002) as presented in Figure 4-4.

4.3.3.1.4 Delta changes in HBI scores from week 0 to week 14

The median change in the HBI scores represented a response in both groups, defined by a drop in a score of 3 points or more (Figure 4-4). The change was similar between normal vitamin D group (-4.0, IQR: -5.3- -2.0) and the low vitamin D group (-3.0, IQR: -6.3 – -1.0), (p=0.49). Individual HBI scores at week 0 and week 14 are presented in Figure 4-5.

A. Low vitamin D group



B. Normal vitamin D group

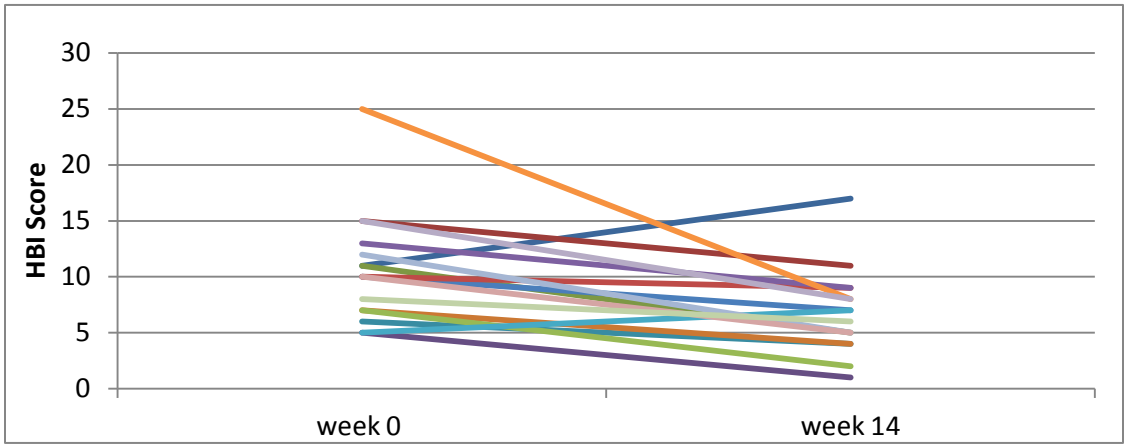


Figure 4-5 Individual HBI scores at week 0 and week 14. Individual HBI scores at week 0 and week 14 in the **(A)** low vitamin D group and **(B)** normal vitamin D group.

4.3.3.2 Partial Mayo scores for ulcerative colitis

Disease activity was assessed in ulcerative colitis patients by the Partial Mayo (PM) questionnaire at weeks 0, 2, 4, 6, and 14. The trend in scores over 14 weeks is presented in Figure 4-6.

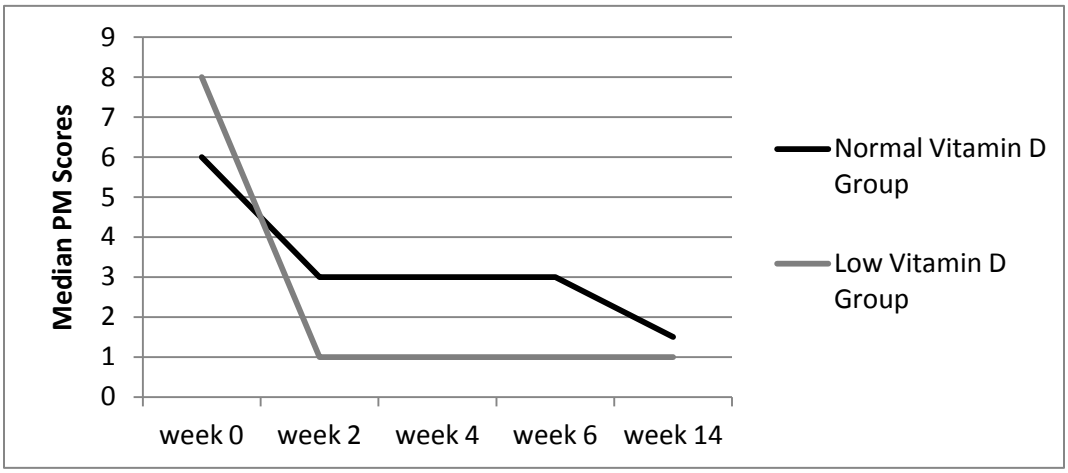


Figure 4-6 PM scores over 14 weeks of anti-TNF therapy. Ulcerative colitis patients had the greatest response at week 2 in both vitamin D groups and maintained this response up to week 14. The low vitamin D group achieved remission by week 2 and maintained remission.

4.3.3.2.1 Baseline PM scores

At baseline, according to the median PM scores, the patients in the normal vitamin D group had moderate disease while the low vitamin D group had severe disease (Figure 4-6). As a result, the low vitamin D group (8.0, IQR: 6.5-9.0) had a slightly higher median PM score than the normal vitamin D group (6.0, IQR: 5.0-8.3), (p=0.18).

4.3.3.2.2 Week 14 PM scores

By week 14, there was a drastic decrease in median PM scores in each group, reflecting improvement in both groups (Figure 4-6). The normal vitamin D group, however, still had a median PM score in the mild disease range (1.5, IQR: 0.0-3.8) and the low vitamin D group reached a median PM score in the remission range (1.0, IQR: 0.0-3.0), (p=0.66).

4.3.3.2.3 Within group-differences between week 14 and week 0 PM scores

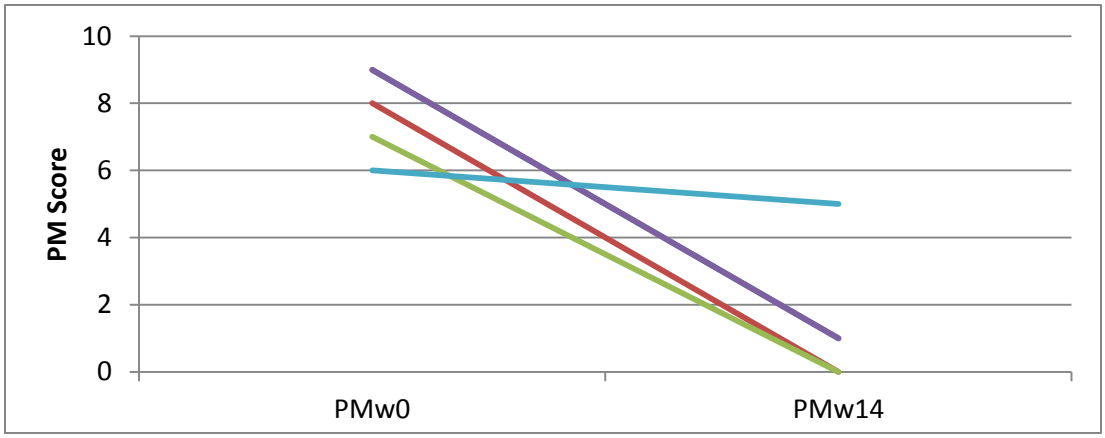
There was a drastic difference between the week 14 median PM score and week 0 median PM score within the normal vitamin D group (1.5, IQR: 0.0-3.8 vs. 6.0, IQR: 5.0 –

8.3, p=0.027) and within the low vitamin D group (1.0, IQR: 0.0-3.0 vs. 8.0, IQR: 6.5-9.0, p=0.039).

4.3.3.2.4 Delta changes in PM scores from week 0 to week 14 Week 14 and Week 0

Similar to the Crohn's disease patients, the median change in the PM scores represented a response in both groups, defined by a drop in a score of 3 points or more (Figure 4-6). There was a large median change from week 0 to week 14 in the low vitamin D group (-8.0, IQR: -8.0- -4.0) and a small change in the normal vitamin D group (-4.5, IQR: -6.3- -2.8), (p=0.13). Individual PM scores at week 0 and week 14 are presented in Figure 4-7.

A. Low vitamin D group



B. Normal vitamin D group

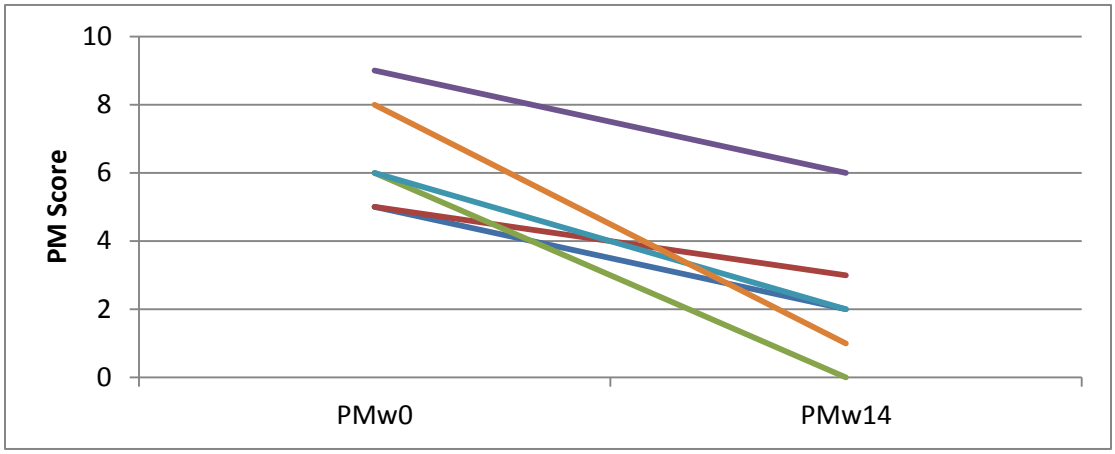


Figure 4-7 Individual PM scores at week 0 and week 14. Individual PM scores of patients in the **(A)** low vitamin D group and **(B)** normal vitamin D group.

4.3.3.3 Clinical Disease Activity Scores, stratified by disease severity

4.3.3.3.1 HBI scores of patients with severe Crohn's disease

At baseline, the median HBI scores were similar between the normal vitamin D group and low vitamin D group (12.0, IQR: 10.0-15.0 vs. 10.0, IQR: 9.0-11.0, p=0.11) in severe Crohn's disease patients. By week 14, the low vitamin D group had a lower median HBI score compared to the normal vitamin D group (5.0, IQR: 3.0-7.0 vs. 9.0, IQR: 7.0-11.0, p=0.028), with similar delta changes from baseline to week 14 in the low vitamin D

group (-6.0, IQR: -8.0 - -4.0) compared to the normal vitamin D group (-4.0, IQR: -6.0 - -2.0), (p=0.36).

4.3.3.3.2 HBI scores of patients with non-severe Crohn's disease

At baseline, median HBI scores were similar between the normal vitamin D group and low vitamin D group (7.0, IQR: 6.0-7.0 vs. 6.0, IQR: 6.0-7.0, p=1.0) in non-severe Crohn's disease patients. By week 14, the normal vitamin D group had a lower median HBI score compared to the low vitamin D group (3.0, IQR: 2.0-4.0 vs. 5.0, IQR: 4.0-6.0, p=0.050) as well as a larger delta change from baseline to week 14 (-4.0, IQR: -5.0- -3.0 vs. -1.0, IQR: -3.0 - -1.0, p=0.05).

4.3.4 Clinical Remission at Week 14

Clinical remission was defined as an achievement of a HBI score less than 5 for patients with Crohn's disease or a PM score of 0 or 1 for patients with ulcerative colitis. Patients who went on to surgery for their disease before week 14 were included and defined as not in remission. Furthermore, patients with an ostomy or who had multiple surgeries had a high 'normal' number of bowel movements, and therefore were excluded. Their 'normal' HBI/PM score may not reach below 5 for Crohn's disease or below 2 for ulcerative colitis; therefore, their disease activity questionnaires could not be used to determine remission.

4.3.4.1 Clinical remission in patients with IBD (including CD and UC)

There were 38 patients with inflammatory bowel disease, including 18 patients in the normal vitamin D group and 20 in the low vitamin D group.

The proportion of patients who achieved clinical remission at week 14 was 33% (6/18) in the normal vitamin D group and 50% (10/20) in the low vitamin D group (p=0.30). There were 27 patients with severe disease, wherein 14% (2/14) of patients in the normal vitamin D group and 62% (8/13) of patients in the low vitamin D group achieved clinical remission at week 14 (p=0.018). Of the 11 patients who had non-severe IBD, 100% (4/4)

in the normal vitamin D group and 29% (2/7) in the low vitamin D group achieved clinical remission at week 14 (p=0.061).

Similar trends were demonstrated among the Crohn's disease and ulcerative colitis patient populations, with a higher proportion of patients with severe disease achieving clinical remission at week 14 in the low vitamin D group and a lower proportion of patients with non-severe disease and low vitamin D achieving remission at week 14 compared to the normal vitamin D group.

4.4 SECONDARY ENDPOINT: EFFICACY AT WEEK 22

4.4.1 Patient Population

Of the 54 patients recruited, there were 32 patients who completed up to week 22 and were included in this week 22 analysis. The 22 patients not included consisted of 10 patients who had not completed up to week 22 by the time of analysis, 6 patients who were withdrawn from the study after week 14 as explained in Figure 4-1, and 6 patients who had fistulizing or stricturing disease. Patients who have had multiple resections or an ostomy were included in this analysis. The number of bowel movements these patients were having was not different from the other patients, and clinical response was still able to be determined. As a result, there were 24 patients with Crohn's disease, with non-stricturing, non-penetrating disease behavior, and 8 ulcerative colitis patients; patients with concomitant perianal disease were included. The demographics of these patients are presented in Table 4.7 and the baseline blood work is presented in Table 4.8. Patients in the low vitamin D group who completed up to week 22 were younger than patients in the normal vitamin D group. All other baseline characteristics were similar.

Table 4-7 Efficacy at week 22: Demographics of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=18)	Low vitamin D Group (n=14)	P-value
Age (years) Median (IQR)	43.5 (30.0-60.0)	30.5 (20.0-38.0)	0.034
Male %, n	50%, 9	64%, 9	0.420
Disease Duration (years) Median (IQR)	5.0 (0.0 – 14.0)	6.5 (1.0-15.0)	0.840
Crohn's Disease %, n	72%, 13	79%, 11	0.680
Disease Location %, n			
Colonic	11%, 2	29%, 4	0.210
Ileal	33%, 6	50%, 7	
Ileocolonic	28%, 5	0%, 0	
Pancolitis	11%, 2	7%, 1	
Left-sided colitis	17%, 3	14%, 2	
Disease Behavior %, n			
Inflammatory	100%, 18	100%, 14	1.00
Penetrating			
Stricturing			
Infliximab %, n	89%, 16	86%, 12	0.790
Smoking Status %, n			0.830
Current	11%, 2	14%, 2	
Nonsmoker	50%, 9	57%, 8	
Former Smoker	39%, 7	29%, 4	
History of Surgery %, n	28%, 5	14%, 2	0.360
Sulfasalazine/mesalamine %, n	28%, 5	21%, 3	1.00
Immunosuppressants %, n	72%, 13	86%, 12	0.360
Corticosteroids %, n	44%, 8	64%, 9	0.270

Table 4-8 Efficacy at week 22: Baseline labs of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Labs	Normal Vitamin D Group (N=18)	Low vitamin D Group (N=14)	P value
Vitamin D (nmol/L) Median (IQR)	92.0 (82.0-102.0)	52.0 (46.0-68.0)	<0.001
Calcium (mmol/L) Median (IQR) Ref. Range 2.10-2.60	2.29 (2.27-2.39)	2.22 (2.17-2.30)	0.095
Albumin (g/L) Median (IQR) Ref. Range: 35-50	40.0 (37.0-43.0)	37.5 (34.0-41.0)	0.079
PTH (pmol/L) Median (IQR) Ref. Range: 1.4-6.8	4.0 (3.4-5.0)	3.1 (2.3-3.3)	0.059
Hemoglobin (g/L) Median (IQR) Ref. Range: 120-160	131.0 (122.0-146.0)	126.0 (110.0-134.0)	0.140
White Blood Cells (10^9/L) Median (IQR) Ref. Range: 4.0-11.0	6.8 (4.9-8.5)	7.0 (5.4-11.9)	0.460
Platelets (10^9/L) Median (IQR) Ref. Range: 140-450	271.0 (213.0-325.0)	306.0 (259.0-406.0)	0.180

4.4.2 Clinical Response at week 14 and week 22

4.4.2.1 Clinical Response in patients with IBD (including CD and UC)

The proportion of patients who responded at week 14 was similar between the normal vitamin D and low vitamin D groups (61% (11/18) vs. 79% (11/14), p=0.29). Furthermore, by week 22, patients in the low vitamin D group maintained their week 14-response of 79% (11/14) after vitamin D supplementation compared to 50% (9/18) of patients in the normal vitamin D group maintaining a clinical response, (p=0.098) (Figure 4-8 A). Patients in the low vitamin D group were 1.6 (95% CI: 0.92-2.7) times more likely to have a clinical response at week 22 than patients in the normal vitamin D group.

4.4.2.2 Clinical response in IBD patients with severe and non-severe disease

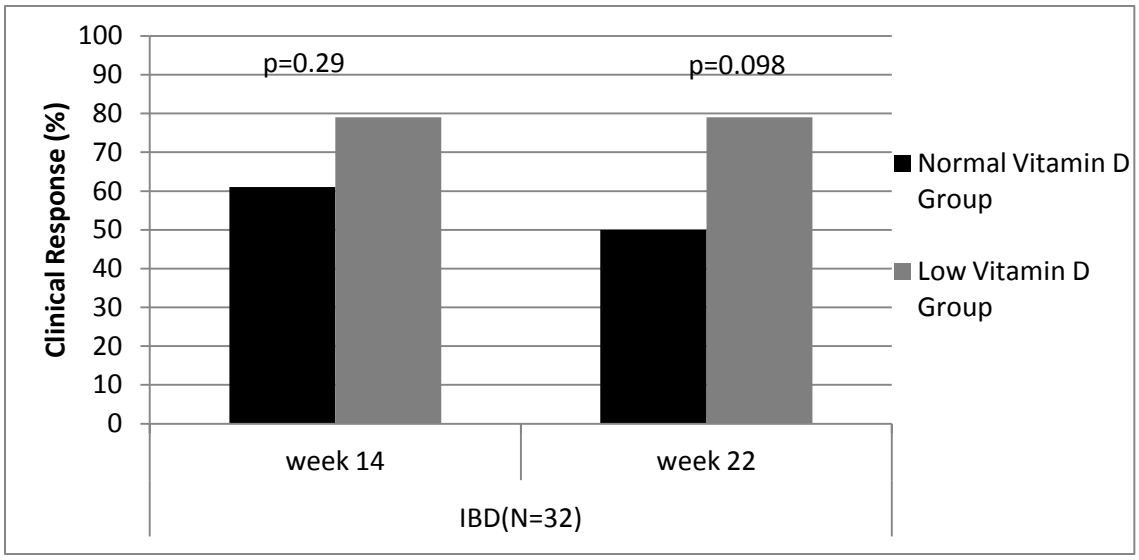
After stratifying by disease severity, there were 23 patients with severe disease and 9 patients with non-severe disease.

Among patients with severe disease, there was a greater proportion of patients with low vitamin D who responded at week 14 (89% (8/9) vs. 50% (7/14), p=0.056; RR: 1.8, 95% CI: 1.0 - 3.2) and week 22 (89% (8/9) vs. 43% (6/14), p=0.027; RR: 2.1, 95% CI: 1.1 - 4.0) compared to the normal vitamin D group with severe disease (Figure 4-8 B). As a result, IBD patients with severe disease and low vitamin D levels before initiating anti-TNF therapy are 1.8 times more likely to clinically respond at week 14 and 2.1 times more likely to clinically respond at week 22 than patients with severe disease and normal vitamin D levels.

In contrast, there was a larger proportion of IBD patients who responded at week 14 in the normal vitamin D group compared to the low vitamin D group, if patients had non-severe disease (100% (4/4) vs. 60% (3/5), p=0.44; RR: 1.7 (95% CI: 0.81-3.4), as presented in Figure 4-8 B. Therefore, IBD patients with non-severe disease and normal vitamin D levels before initiating anti-TNF therapy are 1.7 times more likely to clinically respond at week 14 as patients with non-severe disease and low vitamin D levels.

Furthermore, by week 22, 75% of patients responded in the normal vitamin D group and 60% of patients responded in the low vitamin D group (p=1.0).

A.



B.

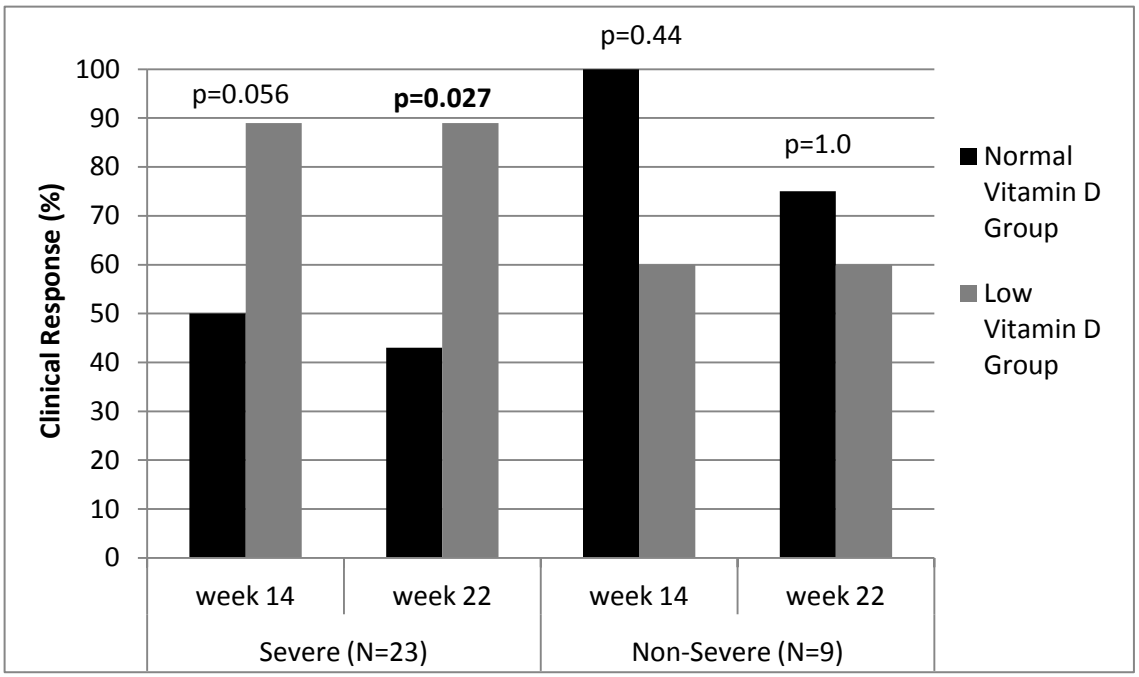


Figure 4-8 Clinical response at week 14 and week 22. A. The proportion of patients with IBD on anti-TNF therapy who responded at week 14 and week 22 stratified by vitamin D status**. B.** The proportion of IBD patients on anti-TNF therapy who responded at week 14 and week 22, stratified by vitamin D status, in the severe-disease and non-severe disease groups. IBD patients with low vitamin D before initiation of anti-TNF therapy have a stronger clinical response at week 14 and week 22, if they have severe disease.

4.4.2.3 Clinical response in patients with Crohn's disease and ulcerative colitis

There were 24 Crohn's disease patients, including 13 patients in the normal vitamin D group and 11 patients in the low vitamin D group, and 8 patients with ulcerative colitis, including 5 patients in the normal vitamin D group and 3 patients in the low vitamin D group.

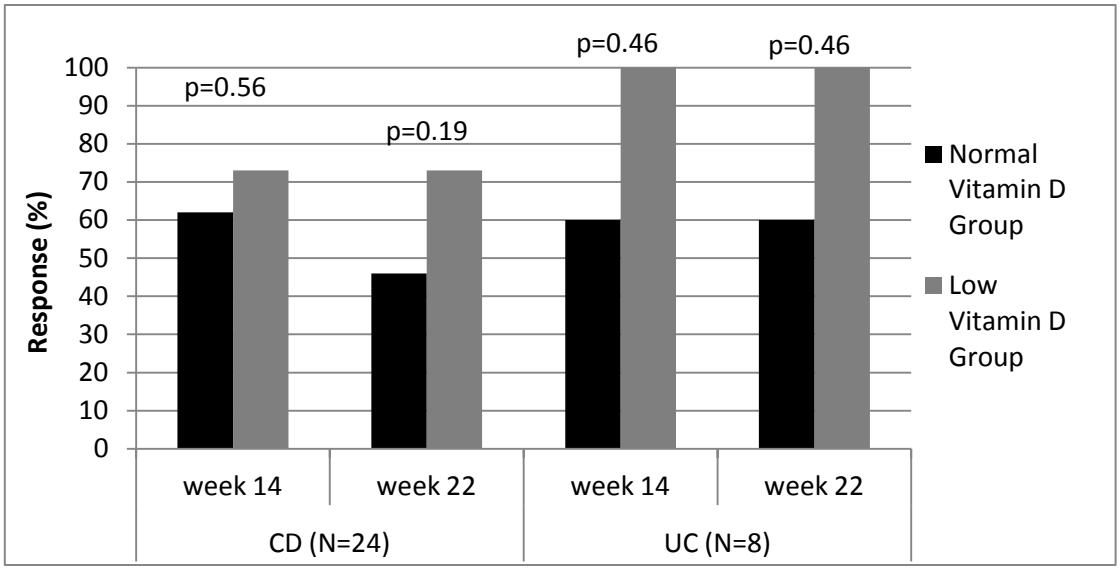
There were similar proportions of patients who responded in the normal and low vitamin D groups of the Crohn's disease population at week 14 (62% (8/13) vs. 73% (8/11), p=0.56; RR: 0.85, 95% CI: 0.48-1.5), with a higher proportion of patients responding at week 22 in the low vitamin D group compared to the normal vitamin D group (46% (6/13) vs. 73% (8/11), p=0.19; RR: 0.63, 95% CI: -.32-1.3). Furthermore, the proportion of patients with ulcerative colitis who responded in the normal vitamin D group was 60% (3/5) and in the low vitamin D group was 100% (3/3), (p=0.46), with a RR of 0.60 (95% CI: 0.29 – 1.2) at week 14, with no change in response rates by week 22. These results are presented in Figure 4-9 A.

4.4.2.4 Clinical response in patients with severe and non-severe CD

After stratifying by disease severity, similar trends as demonstrated in the IBD patients were evident among the Crohn's disease patients. As a result, among patients with severe Crohn's disease, there were 83% (5/6) of patients in the low vitamin D group who clinically responded at week 14 compared to 44% (4/9) of patients in the normal vitamin D group (p=0.29), with similar results at week 22 (83% (5/6) vs. 33% (3/9), p=0.12), as presented in Figure 4-9 B.

In contrast, 100% (4/4) of the non-severe Crohn's disease patients in the normal vitamin D group responded at week 14 compared to 60% (3/5) of the non-severe low vitamin D group at week 14 (p=0.44), with similar trends at week 22 (75% (3/4) vs. 60% (3/5), p=1.0). All the ulcerative colitis patients had severe disease at baseline.

A.



B.

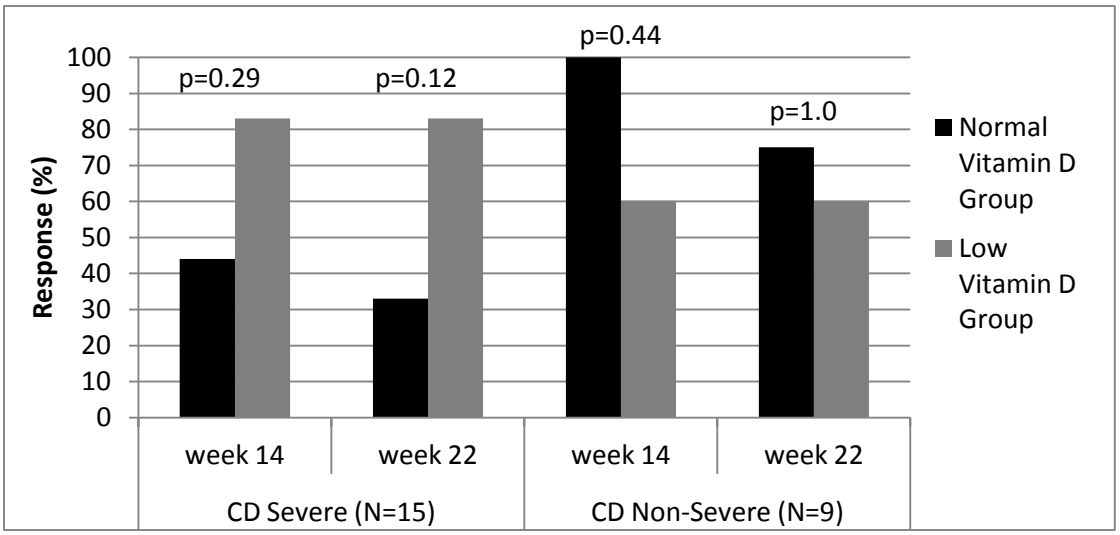


Figure 4-9 Clinical response at week 14 and week 22 in CD and UC patients. **A.** The proportion of patients with Crohn's disease and ulcerative colitis patients on anti-TNF therapy who responded at week 14 and week 22, stratified by vitamin D status. **B.** The proportion of Crohn's disease patients on anti-TNF therapy who responded at week 14 and week 22, stratified by vitamin D status, in the severe-disease and non-severe disease groups. Both Crohn's disease and ulcerative colitis patients who have low vitamin D before initiation anti-TNF therapy seem to have a better response at week 14 and week 22 to therapy, if they had severe disease.

4.4.3 Clinical Disease Activity Scores at Week 14 and week 22

4.4.3.1 Harvey Bradshaw Index for Crohn's Disease

Disease activity was assessed in Crohn's disease patients by the Harvey Bradshaw Index (HBI) questionnaire at weeks 0, 2, 4, 6, 14, 18, and 22. The trend in scores over 22 weeks is presented in Figure 4-10.

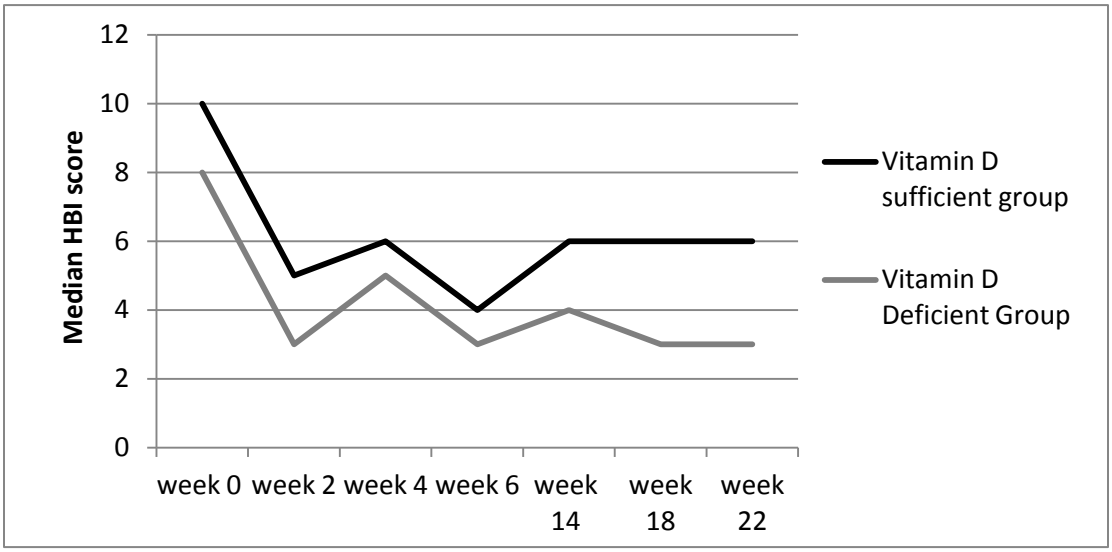


Figure 4-10 HBI scores over 22 weeks of anti-TNF therapy. The median HBI scores over 14 weeks are similar between the two groups. After week 14, the low vitamin D group continued to have a drop in HBI scores.

4.4.3.1.1 Baseline HBI scores

At baseline, the median HBI scores in both groups represented moderate disease, which ranges from 8-16 (Figure 4-10). The normal vitamin D group had a median HBI of 10.0 (IQR: 7.0-15.0) and the low vitamin D group had a median HBI score of 8.0 (IQR: 6.0-10.0), (p=0.37).

4.4.3.1.2 Week 14 HBI scores

By week 14, the normal vitamin D group achieved a median HBI score of 6.0 (IQR: 4.0 – 11.0), which is in the mild disease range, and the low vitamin D group achieved a median HBI score of 4.0 (IQR: 3.0 -6.0), which is in the disease remission range (p=0.15).

4.4.3.1.3 Week 22 HBI scores

By week 22, there was a decrease in median HBI scores from baseline in each group, reflecting improvement in both groups (Figure 4-10). The normal vitamin D group, however, still had a median HBI score in the mild disease range (6.0, IQR: 2.0-10.0) compared to the low vitamin D group which had a median HBI score in the remission range (3.0, IQR: 2.0-6.0), (p=0.17).

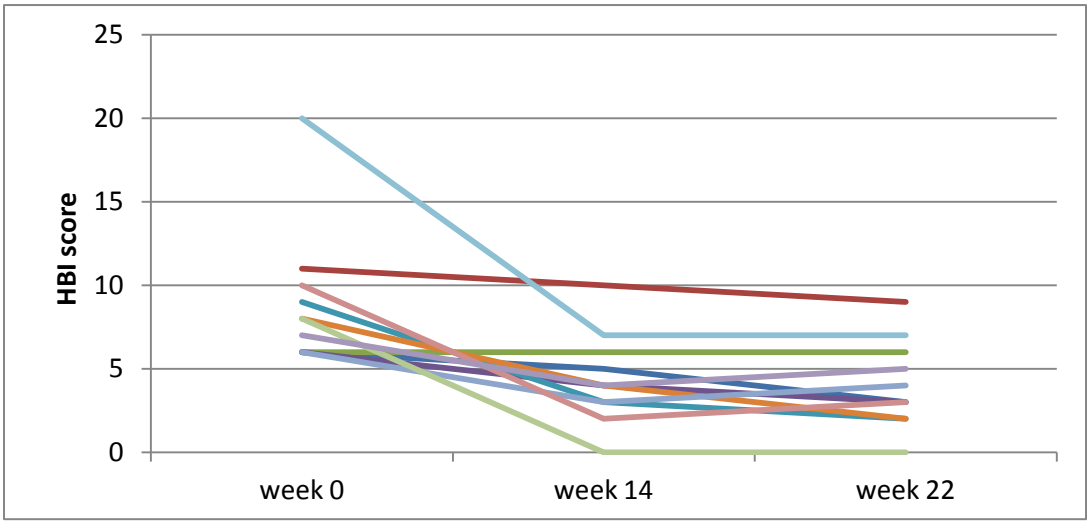
4.4.3.1.4 Within group-differences between week 22 and week 0 HBI scores

The difference between the week 22 median HBI score (6.0, IQR: 2.0-10.0) and week 0 median HBI score (10.0, IQR: 7.0-15.0) within the normal vitamin D group was large (p=0.007). Similar results presented for the low vitamin D group after vitamin D supplementation. There was a large difference between the week 22 score (3.0, IQR: 2.0-6.0) and the week 0 score (8.0, IQR: 6.0-10.0), (p=0.005).

4.4.3.1.5 Delta changes in HBI scores from week 0 to week 22

Interestingly, the median change in the HBI scores from week 0 to week 22 represented a response, in both groups, defined by a drop in a score of 3 points or more. The change was similar between the normal vitamin D group (-4.0, IQR: -5.0 – -1.0) and the low vitamin D group (-3.0 IQR: -7.0 - -2.0) (p=0.56). Similarly, the change from week 0 to week 14 was comparable between the normal vitamin D group (-4.0, IQR:-5.0 - -2.0) and low vitamin D group (-3.0, IQR: -8.0 - -1.0); therefore, there was a median change of 0.0 (IQR: -3.0 – 1.0) in the normal vitamin D group and -1.0 (IQR: -1.0 – 1.0) in the low vitamin D group after vitamin D supplementation from week 14 to week 22. Individual HBI scores at week 0 and week 22 are presented in Figure 4-11.

A. Low vitamin D group



B. Normal vitamin D group

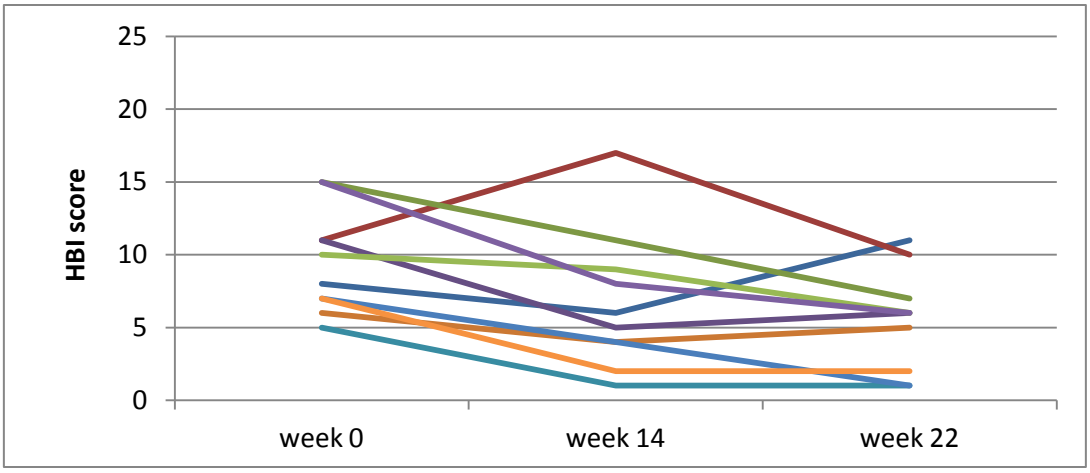


Figure 4-11 Individual HBI scores at week 0, week 14, and week 22 in the **(A)** low vitamin D group and **(B)** normal vitamin D group.

4.4.3.2 Partial Mayo scores for ulcerative colitis

Disease activity was assessed in ulcerative colitis patients by the Partial Mayo (PM) questionnaire at weeks 0, 2, 4, 6, 14, 18 and 22. The trend in scores over 22 weeks is presented in Figure 4-12.

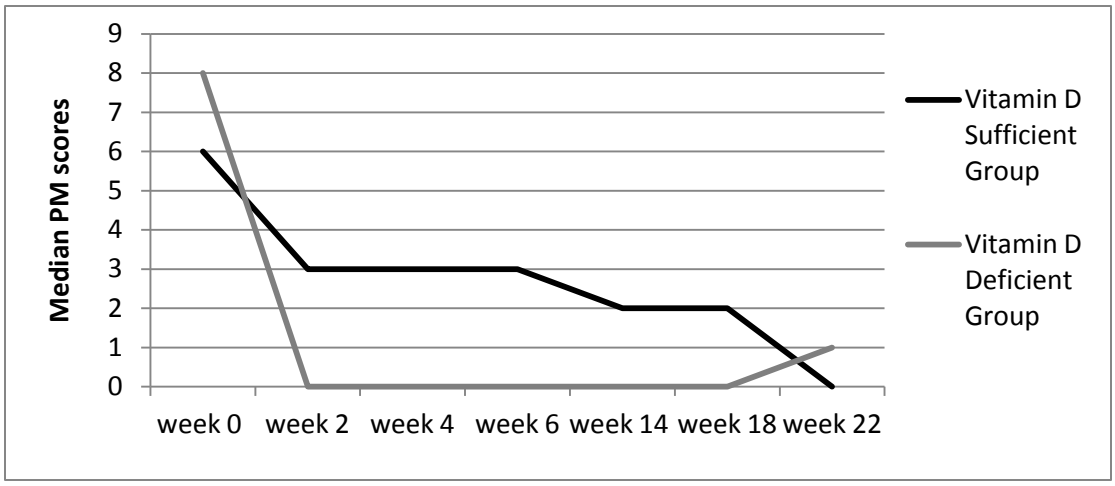


Figure 4-12 PM scores over 22 weeks of anti-TNF therapy. The trend in median PM scores over 22 weeks demonstrated a response in both groups by week 2, which was maintained thereafter. The low vitamin D group demonstrated a faster response, achieving remission by week 14.

4.4.3.2.1 Baseline PM scores

At baseline, according to the median PM scores, the patients in the normal vitamin D group had moderate disease while the low vitamin D group had severe disease (Figure 4-12). As a result, the low vitamin D group (8.0, IQR: 7.0 – 9.0) had a slightly higher median PM score than the normal vitamin D group (6.0, IQR: 6.0-8.0), (p=0.23).

4.4.3.2.2 Week 14 PM scores

By week 14, the normal vitamin D group achieved a median PM score within the mild disease range of 2.0 (IQR: 2.0 – 4.0) and the low vitamin D group achieved a median PM score within the remission range of 0.0 (IQR: 0.0 – 1.0), (p=0.14).

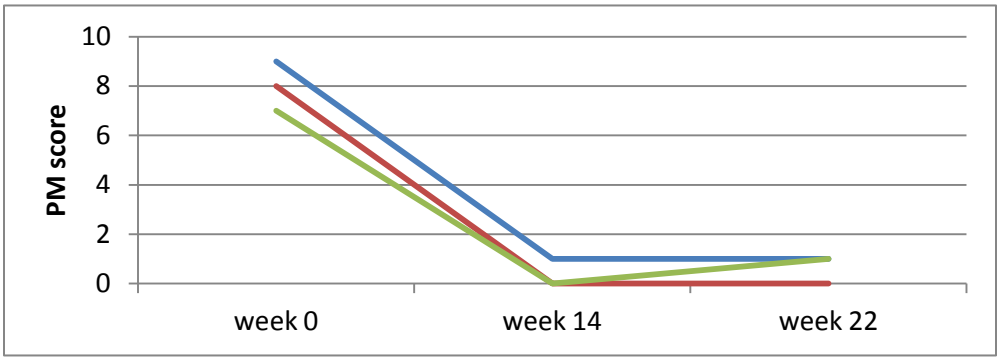
4.4.3.2.3 Week 22 PM scores

By week 22, both groups had similar scores in the clinical remission range, with a median score of 0.0 (IQR: 0.0-2.3) in the normal vitamin D group and 1.0 (IQR: 0.0 -1.0) in the low vitamin D group after vitamin D supplementation (p=0.63).

4.4.3.2.4 Delta changes in PM scores from week 0 to week 22

Similar to the Crohn's disease patients, the median change in the PM scores represented a response in both groups, defined by a drop in a score of 3 points or more (Figure 4-12). The median change from week 0 to week 22 in the low vitamin D group was -8.0 (IQR: -8.0 - -6.0) and the median change in the normal vitamin D group was -6.0 (IQR: -6.0- -5.3), (p=0.11). The median change from week 0 to week 14 was -3.5 (IQR: -5.0 - -3.0) in the normal vitamin D group and -8.0 (IQR: -8.0 –7.0) in the low vitamin D group (p=0.057). As a result, the change from week 14 to week 22 in the normal vitamin D group was -2.00 (IQR: -2.50 - -1.0) and 0.0 (IQR: 0.0 – 1.0) in the low vitamin D group after vitamin D supplementation, (p=0.11). Individual PM scores at week 0, week 14, and week 22 are presented in Figure 4-13.

A. Low vitamin D group



B. Normal vitamin D group

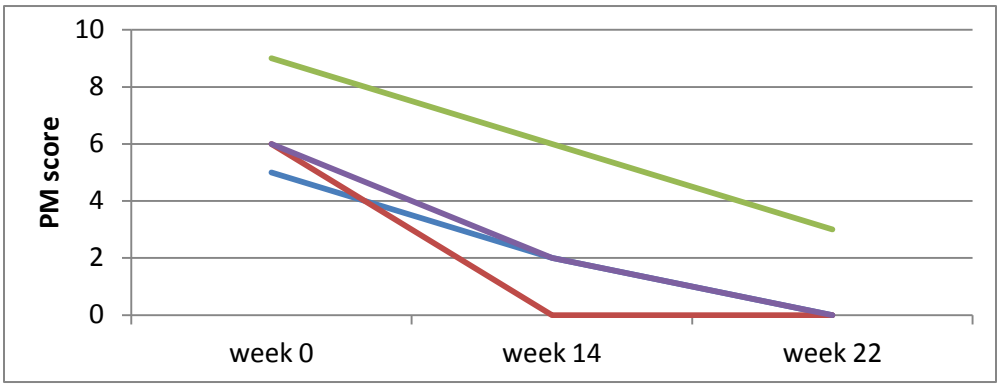


Figure 4-13 Individual PM scores at week 0, week 14, and week 22 in the **(A)** low vitamin D group and **(B)** normal vitamin D group.

4.4.3.3 Clinical Disease Activity Scores, stratified by disease severity

4.4.3.3.1 HBI scores of patients with severe Crohn's disease

At baseline, the median HBI scores were similar between the normal vitamin D group and low vitamin D group (11.0, IQR: 10.0-15.0 vs. 10.0, IQR: 8.0-11.0, p=0.30). By week 22, the low vitamin D group had a lower median HBI score compared to the normal vitamin D group (3.0, IQR: 2.0-7.0 vs. 10.0, IQR: 6.0-11.0, p=0.022), with a trend towards a larger change from baseline to week 22 in the low vitamin D group (-7.0, IQR: -8.0 - -6.0) compared to the normal vitamin D group (-4.0, IQR: -5.0 - -1.0), (p=0.073). Similar trends were demonstrated between the two vitamin D groups from week 0 to week 14, with a similar delta change from week 14 to week 22 in the low vitamin D group after

vitamin D supplementation (-0.5, IQR: -1.0 – 0.0) and normal vitamin D group (1.0, IQR: -1.0 – 2.0), (p=0.84).

4.4.3.3.2 HBI scores of patients with non-severe Crohn's disease

At baseline, the median HBI scores were similar between the normal vitamin D group and low vitamin D group (7.0, IQR: 6.0-7.0 vs. 6.0, IQR: 6.0-6.0, p=1.0). By week 22, the normal vitamin D group had a slightly lower median HBI score compared to the low vitamin D group (2.0, IQR: 1.0-4.0 vs. 4.0, IQR: 3.0-5.0, p=0.11) as well as a slightly larger delta change from baseline to week 22 (-4.5, IQR: -5.5- -2.5 vs. -2.0, IQR: -3.0 - -2.0, p=0.19). Similar trends were demonstrated between the two vitamin D groups from week 0 to week 14, with a similar delta change from week 14 to week 22 in the normal vitamin D group (0.0, IQR: -1.50-0.50) and low vitamin D group after vitamin D supplementation (0.0, IQR: -1.0 – 1.0), (p=0.91).

4.4.4 Clinical Remission At Week 14 and Week 22

Clinical remission was defined as an achievement of a HBI score less than 5 for patients with Crohn's disease or a PM score of 0 or 1 for patients with ulcerative colitis. Patients who went on to surgery for their disease before week 22 were included and defined as not in remission. Furthermore, patients with an ostomy or who had multiple surgeries had a high 'normal' number of bowel movements, and therefore were excluded. Their 'normal' HBI/PM score may not reach below 5 for Crohn's disease or below 2 for ulcerative colitis; therefore, their disease activity questionnaires could not be used to determine remission.

4.4.4.1 Clinical Remission in patients with IBD (including CD and UC)

There were 27 patients with IBD, including 15 patients in the normal vitamin D group and 12 patients in the low vitamin D group.

The proportion of patients who achieved clinical remission at week 14 was 33% (5/15) in the normal vitamin D group and 75% (9/12) in the low vitamin D group (p=0.031). By

week 22, the remission proportion was 40% (6/15) in the normal vitamin D group and 75% (9/12) in the low vitamin D group after vitamin D supplementation(p=0.069).

4.4.4.2 Clinical remission in patients with severe and non-severe IBD

There were 19 IBD patients with severe disease, and 9% (1/11) achieved remission in the normal vitamin D group at week 14 compared to 88% (7/8) in the low vitamin D group (p=0.001). Furthermore, by week 22, 27% (3/11) achieved remission in the normal vitamin D group compared to 88% (7/8) in the low vitamin D group after vitamin D supplementation (p=0.020). Of the 8 patients with non-severe disease, 100% (4/4) achieved clinical remission by week 14 in the normal vitamin D group compared to 50% (2/4) in the low group, as well as 75% (3/4) of the normal vitamin D group achieved remission by week 22, with only 50% (2/4) in the low vitamin D group (p=1.0).

Similar trends were demonstrated among the Crohn's disease and ulcerative colitis population, with a higher proportion of patients with severe disease achieving clinical remission at week 14 and week 22 in the low vitamin D group and a lower proportion of patients with non-severe disease achieving remission at week 14 and week 22 in the low vitamin D group compared to the normal vitamin D group.

4.5 C-REACTIVE PROTEIN RESPONSE AND NORMALIZATION

4.5.1 CRP RESPONSE AND NORMALIZATION AT WEEK 14

Of the 54 patients who completed up to week 14, there were 40 patients who produce CRP and were determined as 'CRP makers'. Of these 40 patients, 5 patients were excluded from the week 14 CRP analysis as there was no result obtained from the week 14-blood work or the patient went on to surgery before week 14 and a CRP level at week 14 was not obtained. Therefore, the total number of IBD patients analyzed was 35, including 30 patients with Crohn's disease and 5 patients with ulcerative colitis.

4.5.1.1 Patient Population

There were 35 IBD patients analyzed, with 15 patients in the normal vitamin D group and 20 in the low vitamin D group. Baseline characteristics were similar between the two groups. Patient demographics are presented in Table 4.9 and baseline blood work is presented in Table 4.10.

Table 4-9 CRP at week 14: Demographics of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=15)	Low vitamin D Group (n=20)	P-value
Age (years) Median (IQR)	31.0 (27.0 – 48.0)	30.5 (22.3 – 38.3)	0.420
Male %, n	40%, 6	70%, 14	0.076
Disease Duration (years) Median (IQR)	3.0 (0.0-9.0)	7.0 (0.25-17.3)	0.250
Crohn's Disease %, n	80%, 12	90%, 18	0.400
Disease Location %, n			
Colonic	13%, 2	40%, 8	0.380
Ileal	40%, 6	35%, 7	
Ileocolonic	27%, 4	15%, 3	
Pancolitis	13%, 2	10%, 2	
Left-sided colitis	7%, 1	0%, 0	
Disease Behavior %, n			
Inflammatory	74%, 11	80%, 16	0.680
Penetrating	13%, 2	5%, 1	
Stricturing	13%, 2	15%, 3	
Infliximab %, n	93%, 14	95%, 19	0.830
Smoking Status %, n			0.540
Current	33%, 5	25%, 5	
Nonsmoker	27%, 4	45%, 9	
Former Smoker	40%, 6	30%, 6	
History of Surgery %, n	20%, 3	20%, 4	1.00
Sulfasalazine/mesalamine %, n	15%, 3	20%, 3	1.00
Immunosuppressants %, n	80%, 12	70%, 14	0.500
Corticosteroids %, n	60%, 9	55%, 11	0.770

Table 4-10 CRP at week 14: Baseline labs of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Labs	Normal Vitamin D Group (N=15)	Low vitamin D Group (N=20)	P value
Vitamin D (nmol/L) Median (IQR)	98.0 (87.0 – 107.0)	52.5 (46.5 – 66.5)	<0.001
Calcium (mmol/L) Median (IQR) Ref. Range 2.10-2.60	2.36 (2.27 – 2.42)	2.27 (2.18 – 2.31)	0.040
Albumin (g/L) Median (IQR) Ref. Range: 35-50	42.0 (37.0 – 43.0)	39.5 (34.5 – 42.0)	0.052
PTH (pmol/L) Median (IQR) Ref. Range: 1.4-6.8	3.0 (2.5– 3.7)	3.2 (2.6 – 4.2)	0.780
Hemoglobin (g/L) Median (IQR) Ref. Range: 120-160	136.0 (125.0 – 148.0)	127.0 (103.75 – 137.75)	0.043
White Blood Cells (10^9/L) Median (IQR) Ref. Range: 4.0-11.0	7.3 (6.4 – 9.7)	7.3 (5.6 – 10.0)	0.610
Platelets (10^9/L) Median (IQR) Ref. Range: 140-450	263.0 (228.0 – 431.0)	331.0 (256.8 - 400.5)	0.440

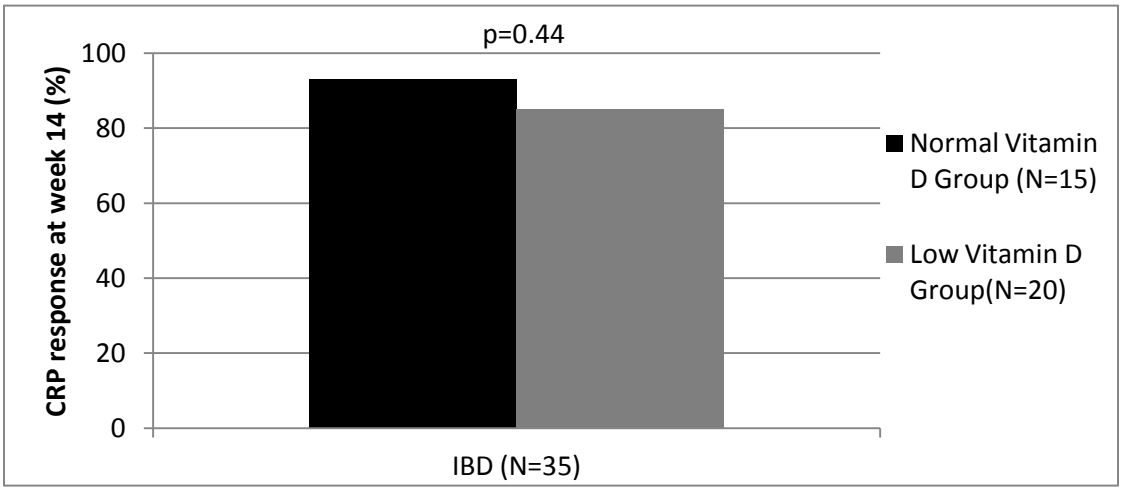
4.5.1.2 CRP response in patients with IBD (including CD and UC)

All patients had a baseline CRP level > 8 mg/L. The median CRP level in the normal vitamin D group was 27.3 mg/L (IQR: 10.8-55.4) and the median CRP level in the low vitamin D group was 24.2 mg/L (IQR: 13.2-58.5), p=0.63. The proportion of patients in

the normal vitamin D group who achieved a CRP response at week 14 was 93% (14/15), defined by a decrease in the patient's baseline CRP level at week 14 by greater than or equal to 50%. Similarly, 85% (17/20) of patients in the low vitamin D group had a CRP response at week 14 (p=0.44), as presented in Figure 4-14 A. The week 14-median CRP levels in both groups were in the normal range < 8 mg/L.

Additionally, there was a similar CRP response at week 14 between the normal vitamin D group and low vitamin D group, if patients had severe disease (88% (7/8) vs. 82% (9/11), p=0.74) or non-severe disease (100% (7/7) vs. 89% (8/9), p=0.36), as presented in Figure 4-14 B.

A.



B.

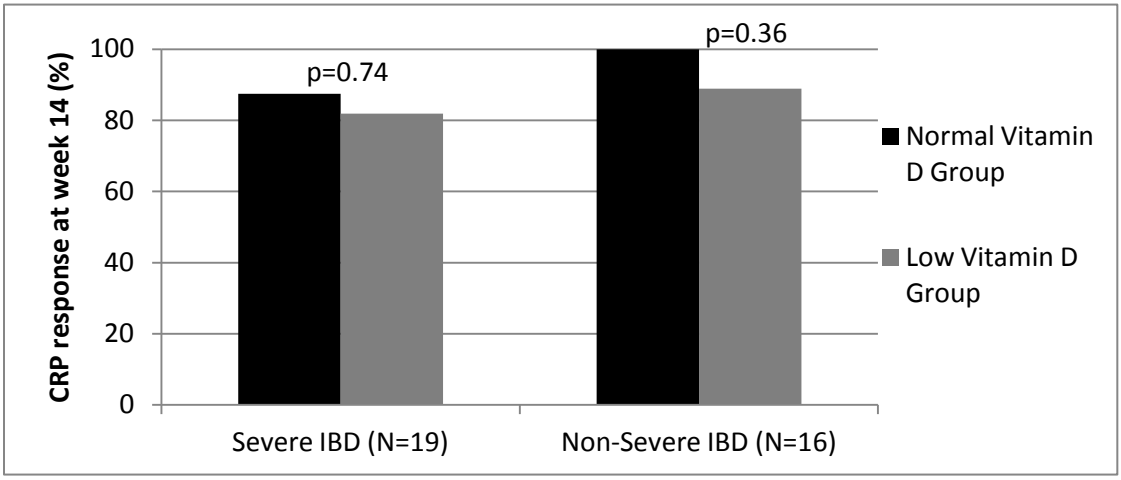


Figure 4-14 CRP response at week 14. A. The proportion of patients with IBD on anti-TNF therapy, who achieved a CRP response at week 14, stratified by vitamin D status. **B.** The proportion of IBD patients on anti-TNF therapy who achieved a CRP response, stratified by vitamin D status in the severe-disease and non-severe disease groups. A similar CRP response was achieved in both groups.

4.5.1.3 CRP Normalization in patients with IBD (including CD and UC)

The proportion of patients in the normal vitamin D group who achieved CRP normalization (CRP level < 8mg/L) at week 14 (80%, 12/15) was similar to the proportion of patients in the low vitamin D group (70%, 14/20), (p=0.50). Furthermore, similar

results presented for patients with severe disease (75% (6/8) vs. 63% (7/11), p=0.60) and non-severe disease (86% (6/7) vs. 79% (7/9), p=0.69).

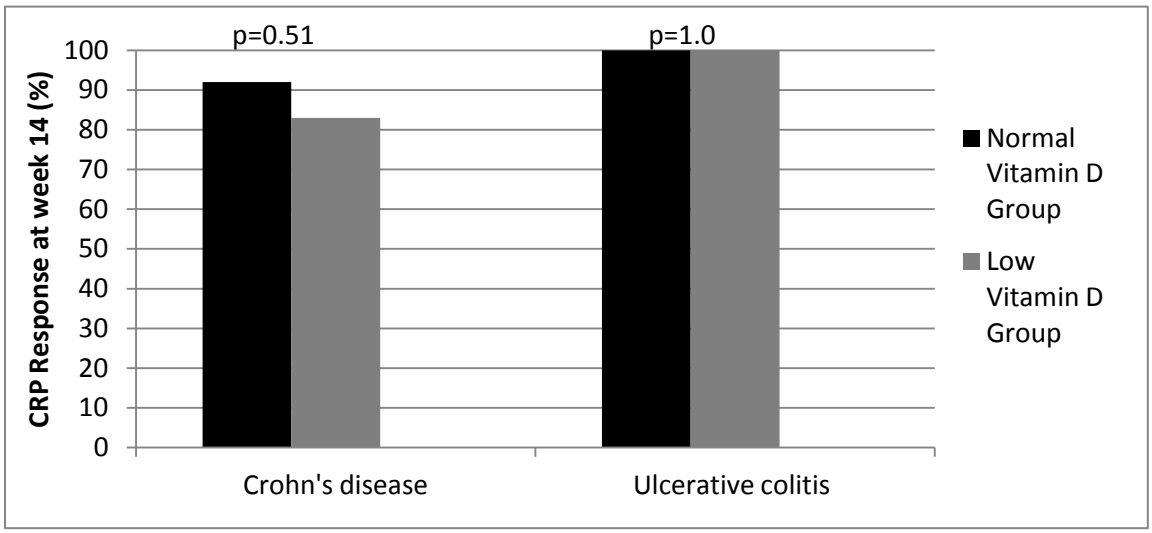
4.5.1.4 CRP response in patients with Crohn's disease and ulcerative colitis

There were 30 patients with Crohn's disease, including 12 patients in the normal vitamin D group and 18 in the low vitamin D group, and 5 patients with ulcerative colitis, including 3 patients in the normal vitamin D group and 2 in the low vitamin D group.

The proportion of Crohn's disease patients in the normal vitamin D group who responded at week 14 was 92% (11/12), which was similar to the 83% (15/18) of patients who responded in the low vitamin D group (p=0.51). Additionally, there was a 100% CRP response in both the normal vitamin D group (3/3) and low vitamin D group (2/2) at week 14 in patients with ulcerative colitis (p=1.0). The results are presented in Figure 4-15 A.

Furthermore, the CRP response at week 14 was similar between the normal vitamin D group and low vitamin D group in patients with severe Crohn's disease (80% (4/5) vs. 78% (7/9), p=1.0) and non-severe Crohn's disease (100% (7/7) vs. 89% (8/9), p=0.36), as presented in Figure 4-15 B. All patients with ulcerative colitis were defined as having severe disease.

A.



B.

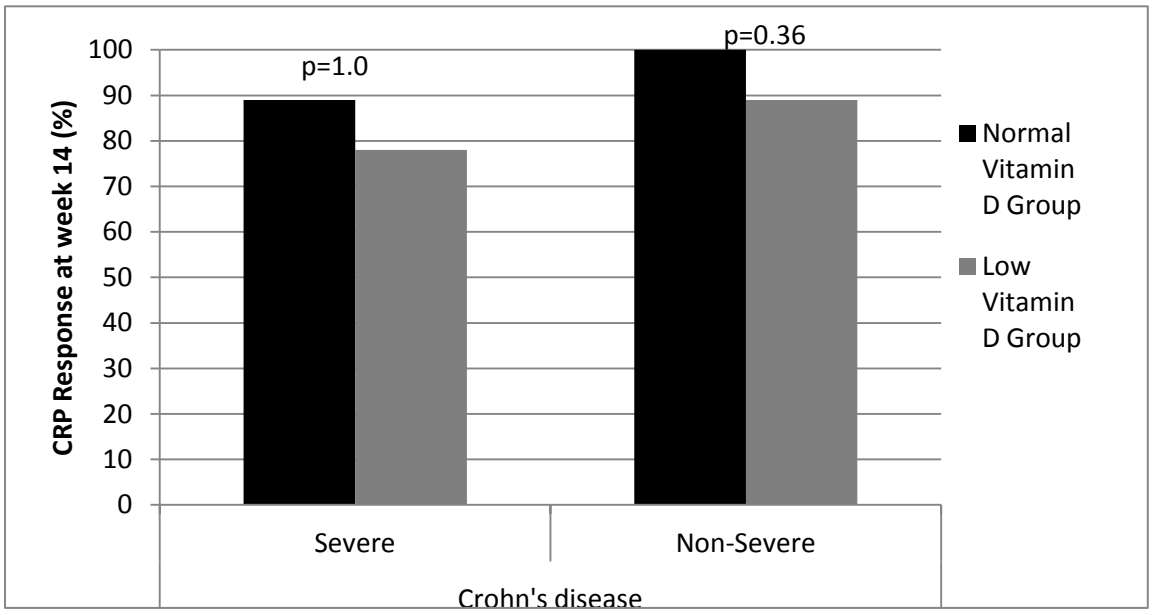


Figure 4-15 CRP response at week 14 in Crohn's disease and ulcerative colitis patients. **A.** The proportion of patients with Crohn's disease and ulcerative colitis on anti-TNF therapy, who achieved a CRP response at week 14, stratified by vitamin D status. **B.** The proportion of patients with Crohn's disease who achieved a CRP response at week 14, stratified by vitamin D status, in the severe-disease and non-severe disease groups. A similar CRP response was achieved in both groups.

4.5.1.5 CRP normalization in patients with Crohn's disease and ulcerative colitis

The proportion of Crohn's disease patients achieved CRP normalization at week 14 was 83% (10/12) in the normal vitamin D group and 67% (12/18) in the low vitamin D group (p=0.31). Furthermore, the proportion of patients with ulcerative colitis who achieved CRP normalization at week 14 was similar between the normal vitamin D group and low vitamin D group (67%, (2/3) vs. 100%, (2/2), p=1.0).

After separating the Crohn's disease patients by disease severity, the proportion of patients with a normalized CRP level at week 14 in the normal vitamin D group was 80% (4/5) and 56% (5/9) in the low vitamin D group (p=0.58). The median CRP levels at baseline were 12.1 mg/L (IQR: 10.5-27.3) in the normal vitamin D group and 22.0 mg/L (IQR: 12.4 – 59.0) in the low vitamin D group, (p=0.30). By week 14, CRP levels were similar (5.2, IQR: 3.4-12.6 vs. 6.1, IQR: 3.8-6.6, p=1.0). There was little difference in the proportion of patients who achieved CRP normalization between the vitamin D groups with non-severe disease (86% (6/7) vs. 78% (7/9), p=0.69).

4.5.2 CRP RESPONSE AND NORMALIZATION AT WEEK 22

Of the 38 patients who completed up to week 22, 8 patients were excluded from the week 22 CRP analysis because these patients had not received their vitamin D injection by week 22 (n=3) or they went on to surgery before week 22 and a CRP level at week 22 was not obtained (n=5). Therefore, the total number of IBD patients analyzed was 25, including 21 patients with Crohn's disease and 4 patients with ulcerative colitis. Of these 25 patients, 3 patients did not have a CRP level measured at week 14. As a result, the week 14 results included 22 patients and the week 22 results included 25 patients.

4.5.2.1 Patient Population

There were 25 patients with IBD, with 11 patients in the normal vitamin D group and 14 in the low vitamin D group who received vitamin D supplementation after week 14. There were more males in the low vitamin D group. All other baseline characteristics

were similar. Patient demographics are presented in Table 4.11 and baseline blood work is presented in Table 4.12.

Table 4-11 CRP at week 22: Demographics of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=11)	Low vitamin D Group (n=14)	P-value
Age (years) Median (IQR)	36.0 (27.0 – 54.0)	30.5 (21.5 – 38.3)	0.220
Male %, n	18%, 2	82%, 9	0.042
Disease Duration (years) Median (IQR)	0.0 (0.0 – 11.0)	7.5 (0.8 – 18.2)	0.200
Crohn's Disease %, n	82%, 9	86%, 12	0.790
Disease Location %, n			
Colonic	18%, 2	29%, 4	0.270
Ileal	28%, 3	50%, 7	
Ileocolonic	36%, 4	7%, 1	
Pancolitis	0%, 0	7%, 1	
Left-sided colitis	18%, 2	7%, 1	
Disease Behavior %, n			
Inflammatory	73%, 8	93%, 13	0.330
Penetrating	18%, 2	7%, 1	
Stricturing	9%, 1	0%, 0	
Infliximab %, n	91%, 10	86%, 12	0.690
Smoking Status %, n			0.270
Current	36%, 4	14%, 2	
Nonsmoker	28%, 3	57%, 8	
Former Smoker	36%, 4	29%, 4	
History of Surgery %, n	9%, 1	21%, 3	0.600
Sulfasalazine/mesalamine %, n	9%, 1	14%, 2	1.00
Immunosuppressants %, n	73%,8	86%, 12	0.420
Corticosteroids %, n	55%, 6	57%, 8	0.900

Table 4-12 CRP at week 22: Baseline labs of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Labs	Normal Vitamin D Group (N=11)	Low vitamin D Group (N=14)	P value
Vitamin D (nmol/L) Median (IQR)	100.0 (920 – 114.0)	52.0 (46.0 – 68.0)	<0.001
Calcium (mmol/L) Median (IQR) Ref. Range 2.10-2.60	2.31 (2.27 – 2.41)	2.25 (2.17– 2.31)	0.120
Albumin (g/L) Median (IQR) Ref. Range: 35-50	40.0 (37.0 – 43.0)	37.0 (33.0 – 41.0)	0.190
PTH (pmol/L) Median (IQR) Ref. Range: 1.4-6.8	3.3 (2.5 – 4.9)	3.2 (2.2 – 4.1)	0.820
Hemoglobin (g/L) Median (IQR) Ref. Range: 120-160	130.0 (121.0 – 151.0)	126.0 (108.0- 1350)	0.290
White Blood Cells (10^9/L) Median (IQR) Ref. Range: 4.0-11.0	8.5 (7.1 – 13.4)	6.6 (5.4 - 10 .7)	0.150
Platelets (10^9/L) Median (IQR) Ref. Range: 140-450	286.0 (252.0 – 459.0)	316.0 (258.3 – 408.5)	0.980

4.5.2.2 CRP response in patients with IBD (including CD and UC)

All patients had a CRP level > 8 mg/L at baseline. The median CRP level at baseline in the normal vitamin D group was 37.3 mg/L (IQR: 15.9-72.6) and the median CRP level at baseline in the low vitamin D group was 29.9 mg/L (IQR: 13.5-61.6), p=0.50. The CRP

response at week 14 in the normal vitamin D group was 100% (10/10) and 75% (9/12) in the low vitamin D group (p=0.089). By week 22, the proportion of patients who achieved a CRP response was 91% (10/11) in the normal vitamin D group and 93% (13/14) in the low vitamin D group after supplementation (p=0.86). (Figure 4-16 A).

If patients had severe disease, 100% (4/4) of patients achieved a CRP response at week 14 in the normal vitamin D group and 75% (6/8) in the low vitamin D group (p=0.52). Similar results presented for patients with non-severe disease (100% (6/6) vs. 75% (3/4), p=0.40). Furthermore, the CRP response at week 22 was similar between the normal and low vitamin D groups in the severe disease population (80% (4/5) vs. 100% (9/9), p=0.36) and non-severe disease population (100% (6/6) vs. 80% (4/5), p=0.46). These results are presented in Figure 4-16 B.

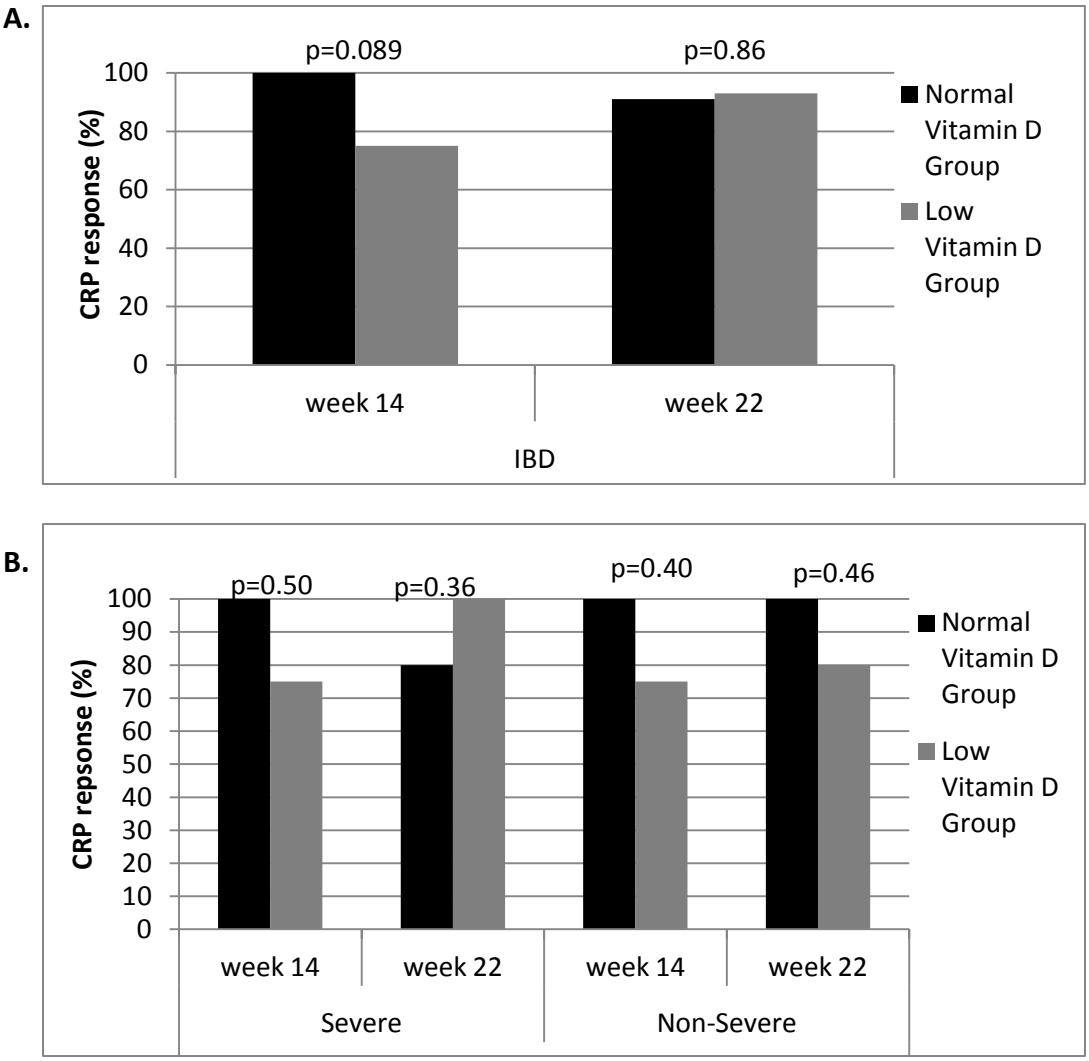


Figure 4-16 CRP response at week 14 and week 22. A. The proportion of patients with IBD who achieved a CRP response at week 14 and week 22, stratified by vitamin D status. Among patients in the low vitamin D group, a higher proportion of patients achieved a CRP response at week 22 compared to week 14 (93% vs. 75%, p=0.02). **B**. The proportion of patients with severe and non-severe IBD who achieved a CRP response at week 14 and week 22, stratified by vitamin D status. Among patients with low serum vitamin D and severe disease, a higher proportion of patients achieved a CRP response at week 22 compared to week 14 (100% vs. 75%, p=0.03).

4.5.2.3 CRP normalization in patients with IBD (including CD and UC)

By week 14, 90% (9/10) of the IBD patients in the normal vitamin D group and 58% (7/12) in the low vitamin group achieved a normal CRP level (p=0.097). Median CRP levels at week 14 were similar between the groups (3.5, IQR: 2.0-6.1 vs. 5.6, IQR: 3.1-12.8, respectively p=0.35). Moreover, by week 22, 55% (6/11) of patients in the normal vitamin D group achieved CRP normalization, wherein 79% (11/14) patients in the low vitamin D group after vitamin D supplementation achieved CRP normalization (p=0.20). Median CRP levels at week 22 were similar between the two groups (4.9, IQR: 1.4-11.4 vs. 2.1, IQR: 0.4-6.6, respectively p=0.095).

Similar results presented for patients with severe disease at week 14 (100% (4/4) vs. 50% (4/8), p=0.21) and week 22 (20% (1/5) vs. 78% (7/9), p= 0.091). The median CRP levels in the severe-normal vitamin D group and severe-low vitamin D group at week 14 were 5.8 mg/L (IQR: 4.6-6.4) and 7.1 mg/L (IQR: 1.9-12.8), respectively (p=0.93). Interestingly, the median CRP level at week 22 in the severe-normal vitamin D group was greater than 8 mg/L and was drastically higher than the median level in the severe-low vitamin D group after vitamin D supplementation (11.4 mg/L , IQR: 9.8 – 14.1 vs. 2.4 mg/L, IQR: 0.40-6.6, respectively p=0.012). There were similar CRP normalization rates in patients with non severe-disease at week 14 (83% (5/6) vs. 75% (3/4), p=1.00) and week 22 (83% (5/6) vs. 80% (4/5), p=1.0).

4.5.2.4 CRP response in patients with Crohn's disease and ulcerative colitis

There were 21 Crohn's disease patients analyzed, with 9 patients in the normal vitamin D group and 12 in the low vitamin D group who achieved normal vitamin D levels by week 22 after supplementation at week 14. There were only 4 patients with ulcerative colitis; therefore, due to the small sample size these patients were not analyzed. The results below are only reflective of patients with Crohn's disease.

4.5.2.4.1 CRP response

The proportion of patients with Crohn's disease who achieved a CRP response by week 14 was 100% (9/9) in the normal vitamin D group and 73% (8/11) in the low vitamin D group (p=0.089). By week 22, 89% (8/9) of the normal vitamin D group had a CRP response and 92% (11/12) of the low vitamin D group after vitamin D supplementation achieved a CRP response, (p=0.83).

Furthermore, the proportion of Crohn's disease who achieved a CRP response at week 14 was similar between the normal and low vitamin D groups if patients had severe disease (100% (3/3) vs. 71% (5/7), respectively p=1.0) as well as if patients had non-severe disease (100% (6/6) vs. 60% (3/5), p=0.18). Additionally, achievement of a CRP response at week 22 was similar between the normal and low vitamin D groups in the severe disease population (67% (2/3) vs. 100% (7/7), p=0.36) and non-severe disease population (100% (6/6) vs. 80% (4/5), p=0.46). These results are presented in Figure 4-17.

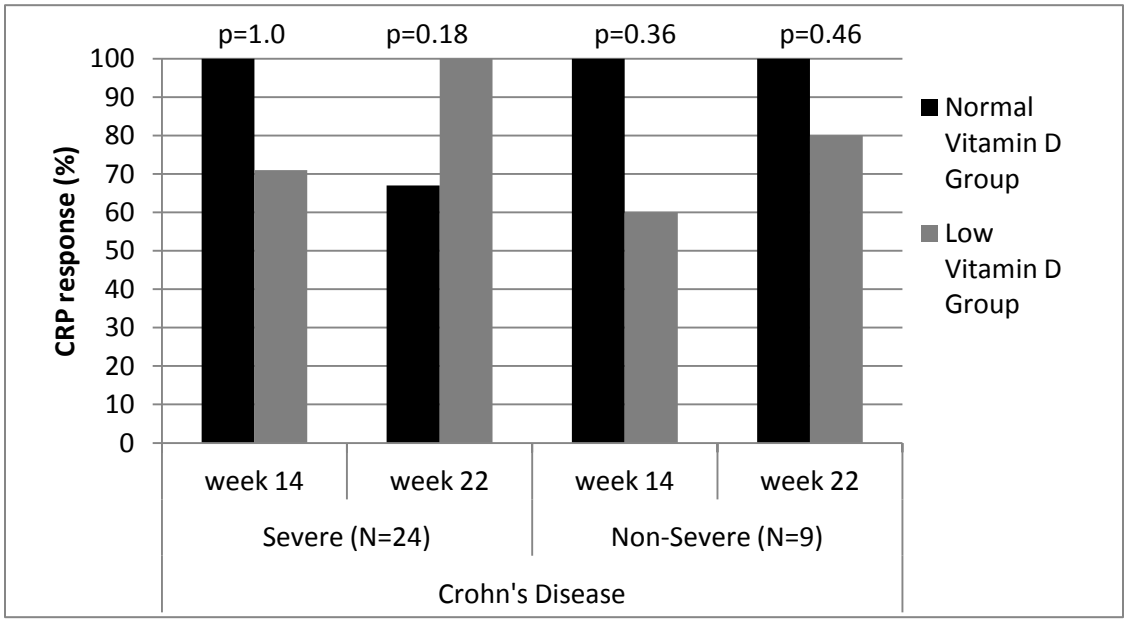


Figure 4-17 CRP response at week 14 and week 22 in severe and non-severe Crohn's disease patients, stratified by vitamin D status.

4.5.2.4.2 CRP Normalization

By week 14, 89% (8/9) of the Crohn's disease patients in the normal vitamin D group achieved CRP normalization compared to 55% (6/11) in the low vitamin D group (p=0.095). By week 22, the proportion of patients in the normal vitamin D group who achieved CRP normalization at week 22 was 56% (5/9) compared to 75% (9/12) of patients in the low vitamin D group after vitamin D supplementation, (p=0.35). Median CRP levels were similar at baseline, week 14, and week 22 between the two groups (data not shown).

Similar results presented for Crohn's disease patients with severe disease at week 14 (100% (3/3) vs. 43% (3/7), p=0.20). The median CRP levels at week 14 were 6.1 mg/L (IQR: 3.8-6.6) in the normal vitamin D group and 9.0 mg/L (IQR: 3.6-13.0) in the low vitamin D group, p=0.67. By week 22, 71% (5/7) of patients in the low vitamin D group achieved CRP normalization after supplementation with 0% (0/3) in the normal vitamin D group (p=0.17). This parallels the median CRP levels at week 22 of 11.4 mg/L (IQR: 9.8-14.1) in the normal vitamin D group and 3.2 mg/L (IQR: 1.8-8.0) in the low vitamin D group after supplementation (p=0.033).

4.6 Influence of Vitamin D on Cytokine Responses

4.6.1 Cytokine responses at week 14

There were 28 patients with IBD who had blood samples collected at week 0 and week 14 for cytokine analysis, with 15 patients in the normal vitamin D group and 13 in the low vitamin D group. Baseline characteristics were similar between the two groups. Patient demographics are presented in Table 4.13 and baseline blood work is presented in Table 4.14.

Table 4-13 Cytokine profiles at week 14: Demographics of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=15)	Low vitamin D Group (n=13)	P-value
Age (years) Median (IQR)	46.0 (30.0-54.0)	28.0 (22.0-36.0)	0.065
Male %, n	53%, 8	46%, 6	0.710
Disease Duration (years) Median (IQR)	5.0 (0.0 – 14.8)	6.5 (0.8 – 15.8)	0.440
Crohn's Disease %, n	73%, 11	69%, 9	0.810
Disease Location %, n			
Colonic	13%, 2	31%, 4	0.690
Ileal	40%, 6	24%, 3	
Ileocolonic	20%, 3	15%, 2	
Pancolitis	7%, 1	15%, 2	
Left-sided colitis	20%, 3	15%, 2	
Disease Behavior %, n			
Inflammatory	87%, 13	77%, 10	0.530
Penetrating	0%, 0	8%, 1	
Stricturing	13%, 2	15%, 2	
Infliximab %, n	100%, 15	100%, 13	1.00
Smoking Status %, n			0.690
Current	20%, 3	31%, 4	
Nonsmoker	53%, 8	54%, 7	
Former Smoker	27%, 4	15%, 2	
History of Surgery %, n	27%, 4	39%, 5	0.690
Sulfasalazine/mesalamine %, n	27%, 4	23%, 3	1.00
Immunosuppressants %, n	80%, 12	77%, 10	0.840
Corticosteroids %, n	40%, 6	62%, 8	0.260

Table 4-14 Cytokine profiles at week 14: Baseline labs of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Labs	Normal Vitamin D Group (N=15)	Low vitamin D Group (N=13)	P value
Vitamin D (nmol/L) Median (IQR)	91.0 (81.0-100.0)	50.0 (46.0-63.0)	<0.001
Calcium (mmol/L) Median (IQR) Ref. Range 2.10-2.60	2.29 (2.22-2.41)	2.28 (2.22-2.32)	0.870
Albumin (g/L) Median (IQR) Ref. Range: 35-50	41.0 (37.0-43.0)	42.0 (35.0-42.0)	0.260
PTH (pmol/L) Median (IQR) Ref. Range: 1.4-6.8	3.7 (2.7-4.1)	3.2 (2.9-4.2)	0.890
Hemoglobin (g/L) Median (IQR) Ref. Range: 120-160	137.5 (121.5-148.5)	124.0 (105.5-130.0)	0.026
White Blood Cells (10^9/L) Median (IQR) Ref. Range: 4.0-11.0	6.4 (4.8-8.4)	8.0 (6.3-11.7)	0.082
Platelets (10^9/L) Median (IQR) Ref. Range: 140-450	275.0 (215.5-343.5)	344.5 (313.0-422.0)	0.029

4.6.1.1 Cytokine levels in patients with IBD (including CD and UC)

At baseline, there were higher levels of IL-6 in the low vitamin D group compared the normal vitamin D group (0.7 pg/ml, IQR: 0.2-1.2 vs. 0.2 pg/ml, IQR: 0.1-0.4, p=0.046). Furthermore, there was a trend towards higher levels of TNF-alpha in the low vitamin D

group compared to the normal vitamin D group (1.6, IQR: 1.5-2.2 vs. 1.3, IQR: 0.6-1.6, p=0.052) (Figure 4-18). However, by week 14, the cytokine levels were similar between the two groups. The changes from week 0 to week 14 within the groups were similar across all cytokines.

4.6.1.2 Cytokine levels in patients with severe and non-severe IBD

There were 22 IBD patients who had severe disease at baseline, with 11 patients in each vitamin D group. Within this group, there were higher levels of IL-1beta, IL-6, and TNF-alpha at baseline in the low vitamin D group compared to the normal vitamin D group (Figure 4-19). However, by week 14, the two groups had similar cytokine levels.

Within the severe-low vitamin D group, IL-6 and TNF-alpha significantly decreased from baseline to week 14. The median level of IL-6 at baseline was 0.7 pg/ml (IQR: 0.2-1.2) compared to 0.1 pg/ml (IQR: 0.08-0.3) at week 14 (p=0.017), and the median TNF-alpha level at baseline was 1.6 pg/ml (IQR: 1.46-2.36) compared to 1.1 pg/ml (0.9-1.3) at week 14 (p=0.037). IL-1beta was not statistically different at week 14 compared to week 0 in the severe-low vitamin D group. Within the severe-normal vitamin D group, levels at baseline were similar to levels at week 14. Furthermore, in the severe-low vitamin D group there was a trend towards a larger decrease in IL-6 (-0.65 pg/ml, IQR: -0.02- -1.35 vs. -0.05, IQR: 0.0- 0.10, p=0.065) and TNF-alpha (-0.54, IQR: -0.04 - -0.74 vs. 0.05, IQR: 0.54 vs. -0.40, p=0.076) compared to the severe-normal vitamin D group.

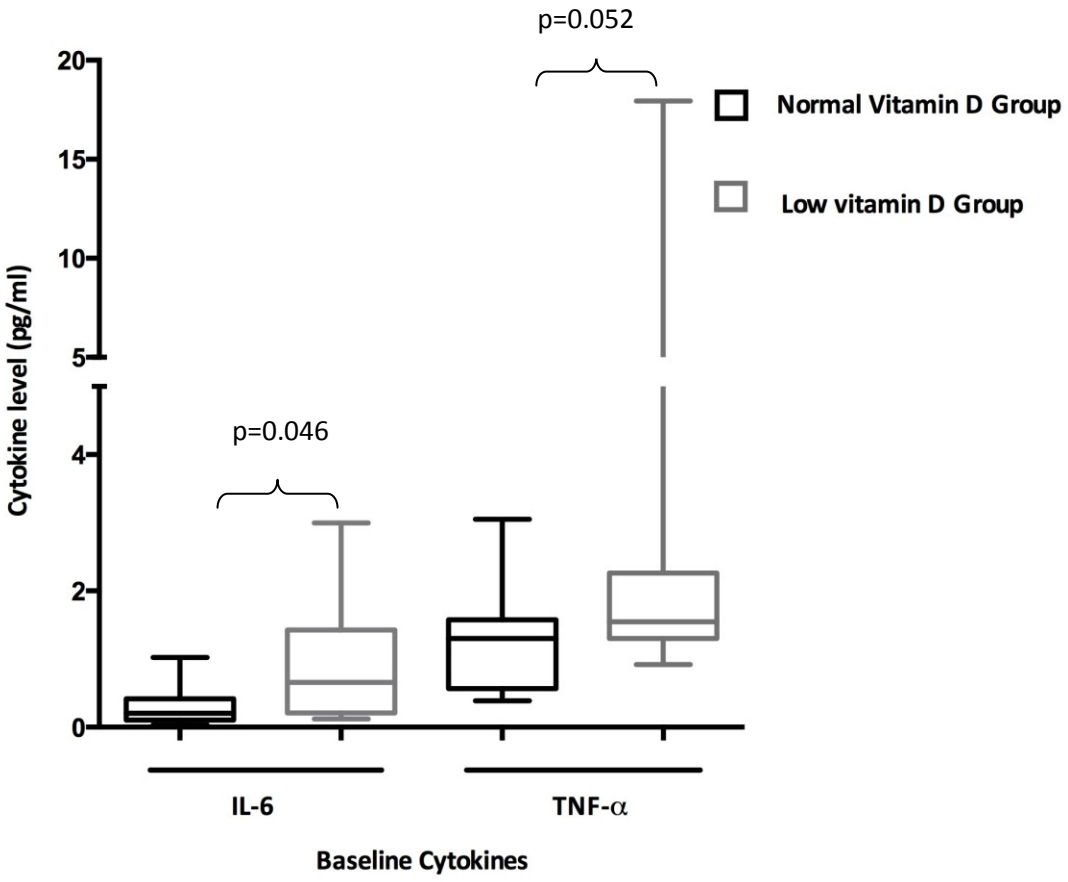


Figure 4-18 Baseline levels of IL-6 and TNF-alpha in IBD patients initiating anti-TNF therapy, stratified by vitamin D status. Baseline IL-6 and TNF-alpha levels were higher in IBD patients who had low vitamin D levels before initiating anti-TNF therapy.

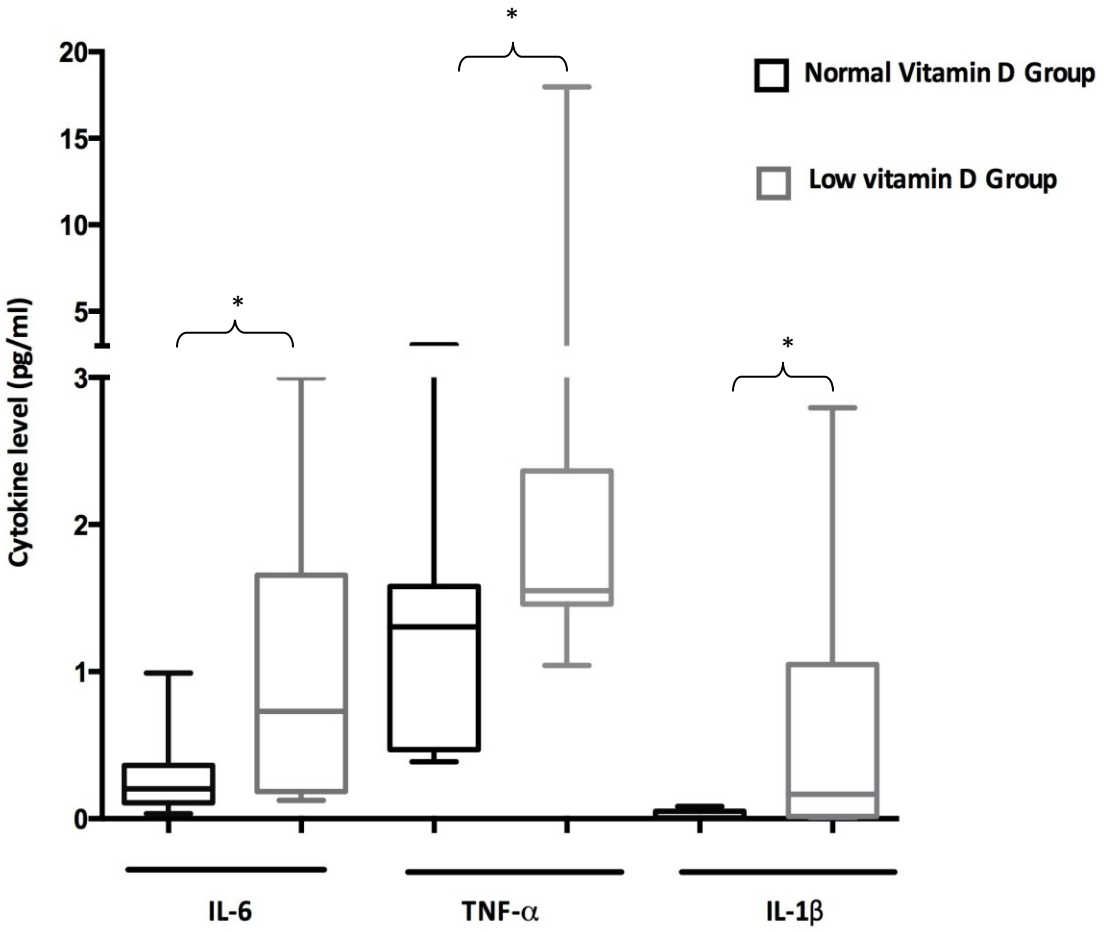


Figure 4-19 Baseline levels of IL-6, TNF-alpha, and IL-beta in IBD patients with severe disease initiating anti-TNF therapy, stratified by vitamin D status. Baseline levels of IL-6, TNF-alpha, and IL-1beta were higher in IBD patients who had low vitamin D levels before initiating anti-TNF therapy (*$p<0.05$).

4.6.2 Cytokine responses at week 22

There were 24 patients who completed up to week 22 and had blood samples collected for cytokine analysis at week 0, week 14, and week 22, with 14 patients in the normal vitamin D group and 10 patients in the low vitamin D group. All baseline characteristics were similar between the groups. The demographics of these patients are presented in Table 4.15 and baseline blood work is presented in Table 4.16.

Table 4-15 Cytokine profiles at week 22: Demographics of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=13)	Low vitamin D Group (n=11)	P-value
Age (years) Median (IQR)	47.0 (32.0-54.0)	36.0 (22.0-38.0)	0.093
Male %, n	46%, 6	36%, 4	0.630
Disease Duration (years) Median (IQR)	13.0 (3.0-20.0)	10.0 (2.0-15.0)	0.530
Crohn's Disease %, n	69%, 9	64%, 7	0.770
Disease Location %, n			
Colonic	15%, 2	27%, 3	0.840
Ileal	40%, 5	9%, 1	
Ileocolonic	15%, 2	28%, 3	
Pancolitis	7%, 1	18%, 2	
Left-sided colitis	23%, 3	18%, 2	
Disease Behavior %, n			
Inflammatory	85%, 11	91%, 10	0.230
Penetrating	0%, 0	9%, 1	
Stricturing	15%, 2	0%, 0	
Infliximab %, n	100%, 13	100%, 11	1.00
Smoking Status %, n			
Current	15%, 2	27%, 3	0.770
Nonsmoker	62%, 8	55%, 6	
Former Smoker	23%, 3	18%, 2	
History of Surgery %, n	23%, 3	36%, 4	0.480
Sulfasalazine/mesalamine %, n	39%, 5	18%, 2	0.390
Immunosuppressants %, n	85%, 11	64%, 7	0.240
Corticosteroids %, n	69%, 9	55%, 6	0.460

Table 4-16 Cytokine profiles at week 22: Baseline labs of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Labs	Normal Vitamin D Group (N=13)	Low vitamin D Group (N=11)	P value
Vitamin D (nmol/L) Median (IQR)	50.0 (29.0-65.0)	91.0 (81.0-100.0)	<0.001
Calcium (mmol/L) Median (IQR) Ref. Range 2.10-2.60	2.29 (2.26-2.40)	2.27 (2.22-2.30)	0.440
Albumin (g/L) Median (IQR) Ref. Range: 35-50	42.0 (39.0-43.0)	39.5 (35.0-41.5)	0.210
PTH (pmol/L) Median (IQR) Ref. Range: 1.4-6.8	3.5 (2.7-4.2)	3.3 (3.1 – 4.2)	0.960
Hemoglobin (g/L) Median (IQR) Ref. Range: 120-160	130.0 (121.0-148.0)	119.5 (102.0-126.0)	0.022
White Blood Cells (10^9/L) Median (IQR) Ref. Range: 4.0-11.0	5.7 (4.6-7.3)	8.1 (5.8-13.0)	0.026
Platelets (10^9/L) Median (IQR) Ref. Range: 140-450	259.5 (213.0– 325.0)	344.5 (306.0-460.0)	0.036

4.6.2.1 Cytokine levels in patients with IBD (including CD and UC)

At baseline, there was a trend towards higher levels of IL-6 and TNF-alpha at baseline in the low vitamin D group compared to the normal vitamin D group. The median level of IL-6 in the low vitamin D group was 0.6 pg/ml (IQR: 0.23-1.66) compared to 0.2 pg/ml

(IQR: 0.13-0.42) in the normal vitamin D group, p=0.074. Furthermore, the median TNF-alpha level in the low vitamin D group was 1.6 pg/ml (IQR: 1.46-2.16) compared to 1.3 pg/ml (IQR: 0.89-1.58) in the normal vitamin D group, p=0.096. The median cytokine levels were similar at week 14 and week 22 between the two groups.

4.6.2.2 Cytokine levels in patients with severe and non-severe IBD

There were 19 patients with severe IBD, including 11 in the normal vitamin D group and 8 in the low vitamin D group. In the severe group of IBD patients, there continued to be a trend towards higher IL-6 and TNF-alpha levels at baseline in the low vitamin D group compared to the normal vitamin D group. Furthermore, there was a trend towards higher levels of IL-1beta in the low vitamin D group at baseline compared to the normal vitamin D group (0.1 pg/ml, IQR: 0.02-1.1 vs. 0.0001 pg/ml, IQR: 0.0001-0.05, p=0.051). The median cytokine levels, however, were similar at week 14 and week 22 between the two groups.

4.6.2.3 Delta changes in cytokines from week 14 to week 22

Although levels of IL-8 at week 22 were similar between the normal vitamin D group (3.5 pg/ml, IQR: 1.73-5.60) and low vitamin D group (2.3 pg/ml, IQR: 1.48-2.91) in IBD patients (p=0.34), the change from week 14 to week 22 in IL-8 levels was different between the two groups, with a larger decrease evident in the low vitamin D group after vitamin D supplementation compared to the normal vitamin D group (-1.2, IQR: -0.05- -3.78 vs. 0.2, IQR: 1.43- -0.24, respectively p=0.036), as presented in Figure 4.20. As a result, within the low vitamin D group, the median IL-8 level at week 22 was significantly lower than this group's IL-8 level at week 14 (p=0.017).

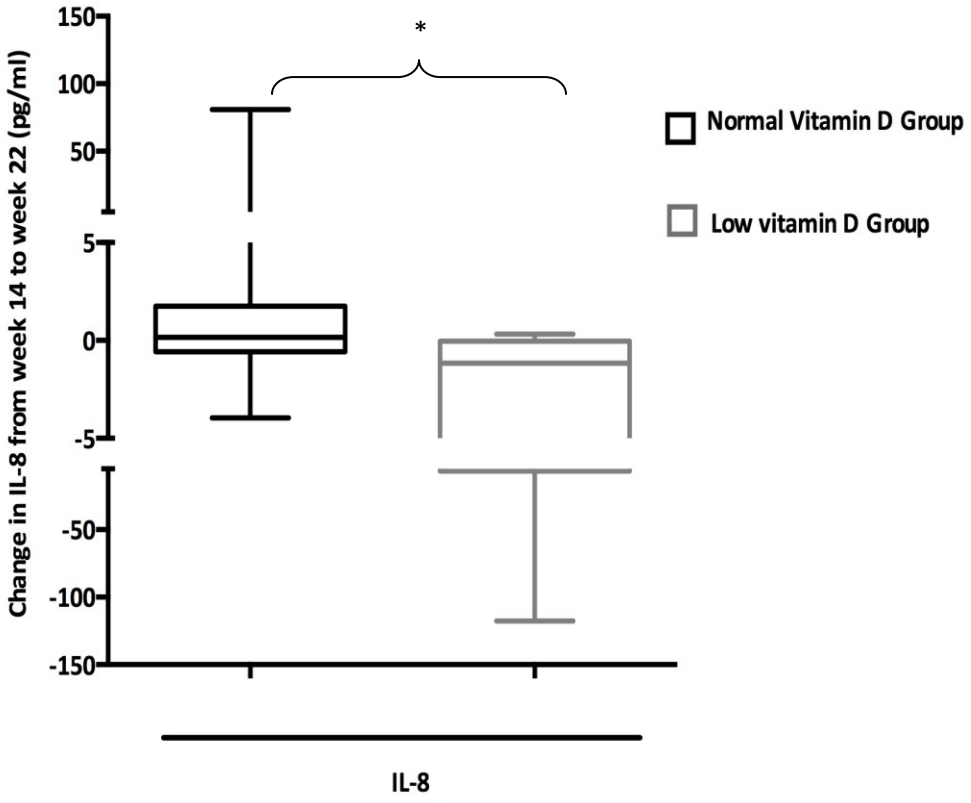


Figure 4-20 Median change in IL-8 from week 14 to week 22 in the normal and low vitamin D (post supplementation) groups. There was a larger decrease in IL-8 in the low vitamin D group after supplementation compared to the normal vitamin D group (*p=0.036).

4.7 The Impact Of Vitamin D Status And Vitamin D Supplementation On Quality of Life In Patients With Inflammatory Bowel Disease On Anti-TNF Therapy

4.7.1 Week 14 Patient Population

There were 54 patients who completed up to week 14 of the study. There were 9 patients excluded from this analysis, as we did not have fully completed questionnaires on these patients at either week 0 or week 14. Of the 9 patients not included, 2 patients went on to surgery before week 14, 3 patients did not complete the questionnaires, and 4 patients did not answer all of the questions of the short inflammatory bowel disease questionnaire (SIBDQ) and therefore, a total score could not be calculated. As a result, there were 45 patients with IBD included in this SIBDQ analysis, with 22 patients in the normal vitamin D group and 23 patients in the low vitamin D group. Patients in the low vitamin D group were younger. All other baseline characteristics were similar. The demographics of these patients are presented in Table 4.17. Furthermore, Table 4.18 presents the distribution of disease severity before anti-TNF therapy initiation among this patient population. There was a similar distribution of patients with mild, moderate and severe disease between the two groups.

Table 4-17 Quality of life at week 14: Demographics of patients with IBD initiating anti-TNF therapy, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=22)	Low vitamin D Group (n=23)	P-value
Age (years) Median (IQR)	40.5 (30.75 – 58.0)	28.0 (21.0 – 38.0)	0.023
Male %, n	50%, 11	74%, 17	0.098
Disease Duration (years) Median (IQR)	8.0 (2.5 – 14.8)	10.0 (2.0 – 19.0)	0.660
Crohn's Disease %, n	77%, 17	83%, 19	0.660
Disease Location %, n			
Colonic	23%, 5	30%, 4	0.970
Ileal	36%, 8	17%, 4	
Ileocolonic	18%, 4	35%, 8	
Pancolitis	14%, 3	9%, 2	
Left-sided colitis	9%, 2	9%, 2	
Disease Behavior %, n			
Inflammatory	86%, 19	83%, 19	0.140
Penetrating	14%, 3	4%, 1	
Stricturing	0%, 0	13%, 3	
Infliximab %, n	91%, 20	96%, 22	0.520
Smoking Status %, n			
Current	32%, 7	22%, 5	0.750
Nonsmoker	41%. 9	48%, 11	
Former Smoker	27%, 6	30%, 7	
History of Surgery %, n	32%, 7	22%, 5	0.450
Sulfasalazine/mesalamine %, n	32%, 7	17%, 4	0.260
Immunosuppressants %, n	73%, 16	74%, 17	0.930
Corticosteroids %, n	41%, 9	65%, 15	0.100

Table 4-18 Quality of life at week 14: Distribution of disease severity of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

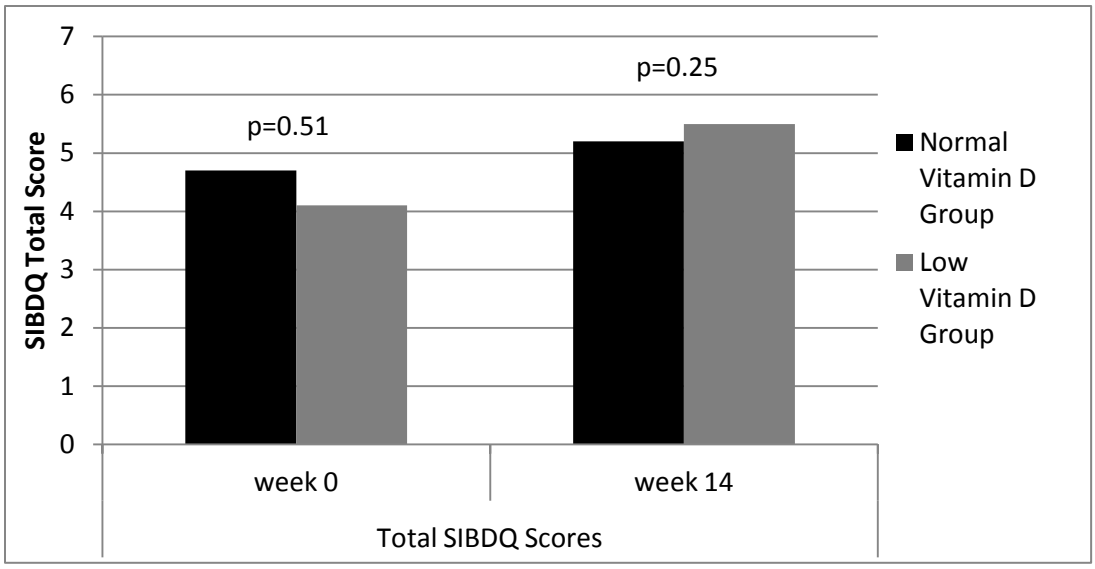
Disease Severity	Week 0		P-value
	Normal Vitamin D Group (n=22)	Low Vitamin D Group (n=23)	0.360
% can't assess	14%	17%	
% mild	18%	35%	
% moderate – severe	68%	48%	

4.7.1.1 Quality of life in patients with IBD (including CD and UC)

Baseline total scores were similar in the normal vitamin D and low vitamin D groups (4.7, IQR: 2.9-5.7 vs. 4.1, IQR: 3.2-4.7, respectively p=0.51). By week 14, scores were similar and improved in both groups (5.2, IQR: 4.6-5.83 vs. 5.5, IQR: 4.9-5.9, p=0.25), as presented in Figure 4-21 A.

Patients with normal and low vitamin D levels had similar scores at baseline and week 14, in both the severe and non-severe disease groups (Figure 4-21 B). There was, however, a larger improvement (increase in scores) from week 0 to week 14 in the severe-low vitamin D group compared to the patients in the severe-normal vitamin D group (1.7, IQR: 0.7-2.6 vs. 0.5, IQR: -0.5-1.3, respectively p=0.047).

A.



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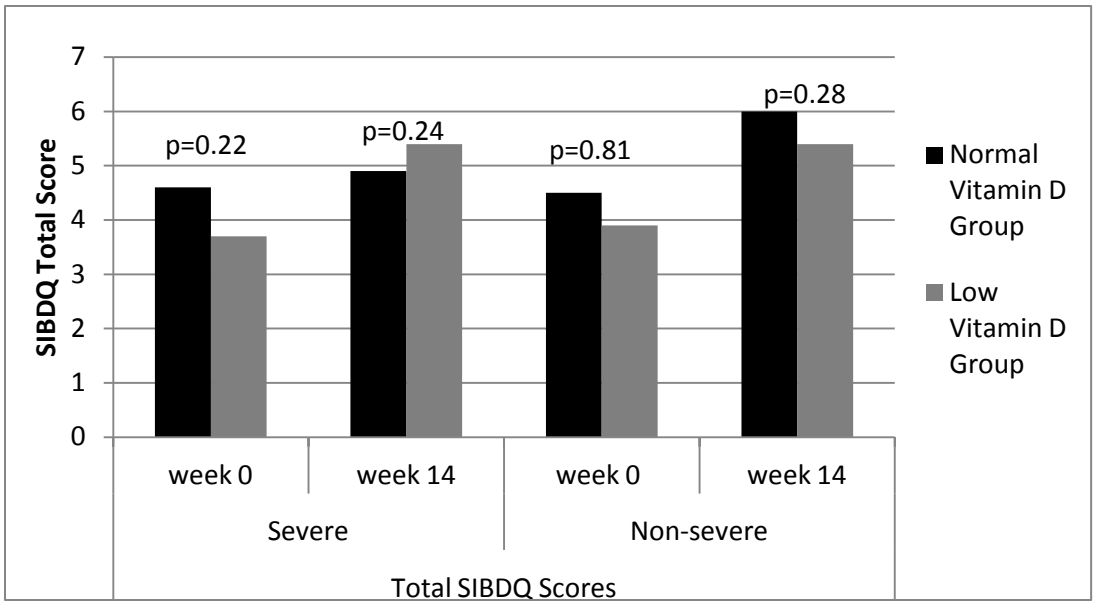


Figure 4-21 Total SIBDQ scores at week 0 and week 14 in patients with IBD on anti-TNF therapy. A. Total quality of life scores in IBD patients on anti-TNF therapy, stratified by vitamin D status. **B.** Total quality of life scores in IBD patients on anti-TNF therapy, stratified vitamin D status, in the severe-disease and non-severe disease groups. Scores are similar between the groups. Among patients with severe disease, there was a larger increase in SIBDQ scores from week 14 to week 22 in the low vitamin D group compared to the normal vitamin D group (1.7, IQR: 0.7-2.6 vs. 0.5, IQR: -0.5-1.3, p=0.047).

4.7.2 Week 22 Patient Population

There were 38 patients who completed up to week 22 of the study. There were 9 patients excluded from this analysis, as we did not have completed data on these patients at either week 0 or week 22. Of the 9 patients not included, 5 patients went on to surgery before week 14, 1 patient did not complete the questionnaire, and 3 patients did not answer all of the questions of the short inflammatory bowel disease questionnaire (SIBDQ) and therefore, a total score could not be calculated. As a result, there were 29 patients with IBD included in this SIBDQ analysis, with 16 patients in the normal vitamin D group and 13 patients in the low vitamin D group. Patients were younger in the low vitamin D group. All other baseline characteristics were similar between the groups. The demographics of these patients are presented in Table 4.19, and the distribution of disease severity of patients before initiating anti-TNF therapy is presented in Table 4.20. There was a similar distribution of patients with mild, moderate and severe disease between the two groups.

Table 4-19 Quality of life at week 22: Demographics of IBD patients initiating anti-TNF, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=16)	Low vitamin D Group (n=13)	P-value
Age (years) Median (IQR)	43.5 (29.8 – 61.0)	27.0 (20.0 – 37.0)	0.020
Male %, n	38%, 6	69%, 4	0.089
Disease Duration (years) Median (IQR)	7.0 (0.0 – 14.0)	8.0 (1.5 – 16.5)	0.850
Crohn's Disease %, n	81%, 13	77%, 10	0.780
Disease Location %, n			
Colonic	19%, 3	31%, 4	0.640
Ileal	31%, 5	38%, 5	
Ileocolonic	31%, 5	8%, 1	
Pancolitis	6%, 1	8%, 1	
Left-sided colitis	13%, 2	15%, 2	
Disease Behavior %, n			
Inflammatory	88%, 14	92%, 12	0.670
Penetrating	12%, 2	8%, 1	
Stricturing	0%, 0	0%, 0	
Infliximab %, n	88%, 14	92%, 12	0.670
Smoking Status %, n			0.620
Current	19%, 3	15%, 2	
Nonsmoker	44%, 7	62%, 8	
Former Smoker	37%, 6	23%, 3	
History of Surgery %, n	25%, 4	23%, 3	1.00
Sulfasalazine/mesalamine %, n	25%, 4	23%, 3	1.00
Immunosuppressants %, n	69%, 11	85%, 11	0.320
Corticosteroids %, n	38%, 6	54%, 7	0.380

Table 4-20 Quality of life week 22: Distribution of disease severity of IBD patients, stratified by vitamin D status

Disease Severity	Week 0		P-value
	Normal Vitamin D Group (n=16)	Low Vitamin D Group (n=13)	0.350
% can't assess	12%	15%	
% mild	25%	31%	
% moderate - severe	63%	54%	

4.7.2.1 Quality of life scores in patients with IBD (including CD and UC)

SIBDQ median scores were similar between the normal vitamin D group and low vitamin D group at baseline (5.3, IQR: 3.3-6.0 vs. 3.8, IQR: 3.6-4.4, p=0.17) as well as at week 14 (5.6, IQR: 4.8-5.9 vs. 5.5, IQR: 4.9-5.9, p=0.93) and week 22 (5.2, IQR: 4.4-6.1 vs. 5.9, IQR: 5.5-6.1, p=0.14). The median change from week 0 to week 14 was larger in the low vitamin D compared to the normal vitamin D group (1.30, IQR: 0.70-1.80 vs. 0.10, IQR: -0.50 – 1.30, p=0.037) as well as from week 14 to week 22 after the low group received vitamin D supplementation (0.40, IQR: 0.00-0.50 vs. 0.00, IQR: -0.30-0.20, p=0.029), as presented in Figure 4.23 A.

Similar scores presented for IBD patients at each time point after stratifying them into severe disease and non-severe disease groups; however, by week 22, there was a trend towards higher SIBDQ scores in the severe-low vitamin D group compared to the severe-normal vitamin D group (5.9, IQR: 5.3-6.4 vs. 4.9, IQR: 3.9-5.9, respectively, p=0.088) and a trend towards a higher median SIBDQ score in the non-severe-normal vitamin D group compared to the non-severe-low vitamin D group (6.1, IQR: 5.9-6.1 vs. 5.5, IQR: 5.1-5.8, respectively, p=0.057), as presented in Figure 4.23 B. The delta change in scores from week 0 to week 14 and week 14 to week 22 were significantly higher in the low-severe vitamin D group compared to the severe-normal vitamin D group. The changes at

these time points in patients with non-severe disease were similar between the normal and low vitamin D groups.

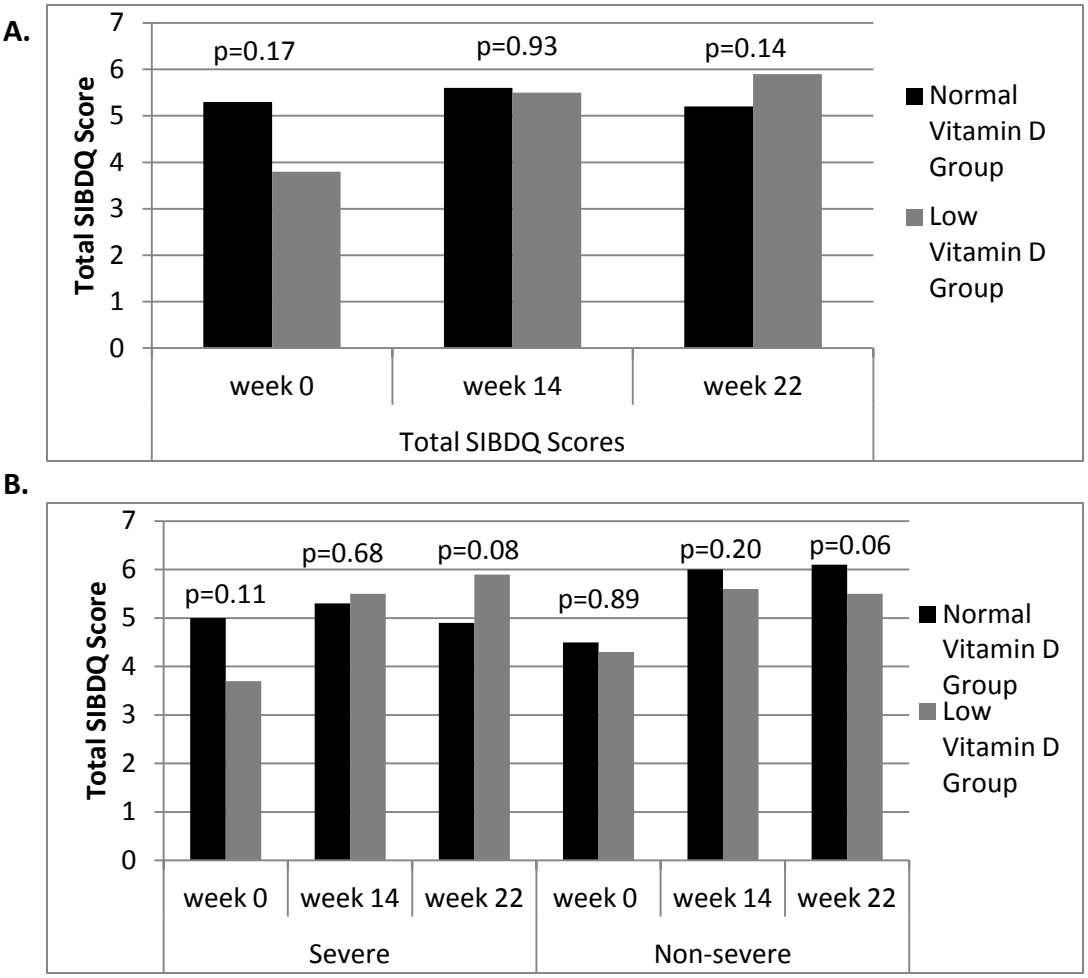


Figure 4-22 Total SIBDQ scores at week 0, week 14, and week 22 in patients with IBD on anti-TNF therapy. A. Total SIBDQ scores in IBD patients, stratified by vitamin D status. There was larger improvement in SIBDQ scores from week 0 to week 14 (p=0.037) and week 14 to week 22 (p=0.029) in the low vitamin D group compared to the normal vitamin D group. **B.** Total SIBDQ scores in IBD patients, stratified by vitamin D status, in the severe-disease and non-severe disease groups. Among patients with severe disease, there was larger improvement in SIBDQ scores from week 0 to week 14 (p=0.031) and week 14 to week 22 (p=0.012) in the low vitamin D group compared to the normal vitamin D group.

4.8 The Impact Of Vitamin D Status And Vitamin D Supplementation On Depression In Patients With Inflammatory Bowel Disease On Anti-TNF Therapy

4.8.1 Week 14 Patient Population

There were 54 patients who completed up to week 14 of the study. Of these patients, there were 8 excluded from this depression analysis, as we did not have completed data on these patients at either week 0 or week 14. Of the 8 patients not included, 2 patients went on to surgery before week 14, 3 patients did not complete the questionnaires, and 3 patients did not answer all of the questions of the BDI-II questionnaire, and therefore, their final score could not be calculated. As a result, there were 46 patients with IBD included in this analysis, with 23 patients in the normal vitamin D group and 23 patients in the low vitamin D group. Patients in the low vitamin D group were younger. All other baseline characteristics were similar. The demographics of these patients are presented in Table 4.21 and the distribution of disease severity of patients before initiating anti-TNF therapy is presented in Table 4.22. There was a similar distribution of patients with mild, moderate and severe disease between the two groups.

Table 4-21 Depression at week 14: Demographics of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=23)	Low vitamin D Group (n=23)	P-value
Age (years) Median (IQR)	41.0 (31.0 – 57.0)	33.0 (22.0 – 38.0)	0.021
Male %, n	44%, 10	70%, 16	0.074
Disease Duration (years) Median (IQR)	7.0 (1.0 – 14.0)	10.0 (2.0 – 19.0)	0.510
Crohn's Disease %, n	73.9% (17)	82.6% (19)	0.480
Disease Location %, n			
Colonic	22%, 5	30%, 7	0.950
Ileal	35%, 8	35%, 8	
Ileocolonic	17%, 4	17%, 4	
Pancolitis	13%, 3	9%, 2	
Left-sided colitis	13%, 3	9%, 2	
Disease Behavior %, n			
Inflammatory	83%, 19	83%, 19	0.370
Penetrating	13%, 3	4%, 1	
Stricturing	4%, 1	13%, 3	
Infliximab %, n	91.3% (21)	91.3% (21)	1.00
Smoking Status %, n			
Current	30%, 7	22%, 5	0.770
Nonsmoker	44%, 10	52%, 12	
Former Smoker	26%, 6	26%, 6	
History of Surgery %, n	26%, 17	22%, 18	0.730
Sulfasalazine/Mesalamine %, n	30.4% (7)	17.4% (4)	0.300
Immunosuppressants %, n	73.9% (17)	73.9% (17)	1.00
Corticosteroids %, n	44%, 10	65% 15	0.140

Table 4-22 Depression at week 14: Distribution of disease severity in IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Disease Severity	Week 0		P-value
	Normal Vitamin D Group	Low Vitamin D Group	0.560
% can't assess	17%	17%	
% mild	17%	30%	
% moderate - severe	65%	52%	

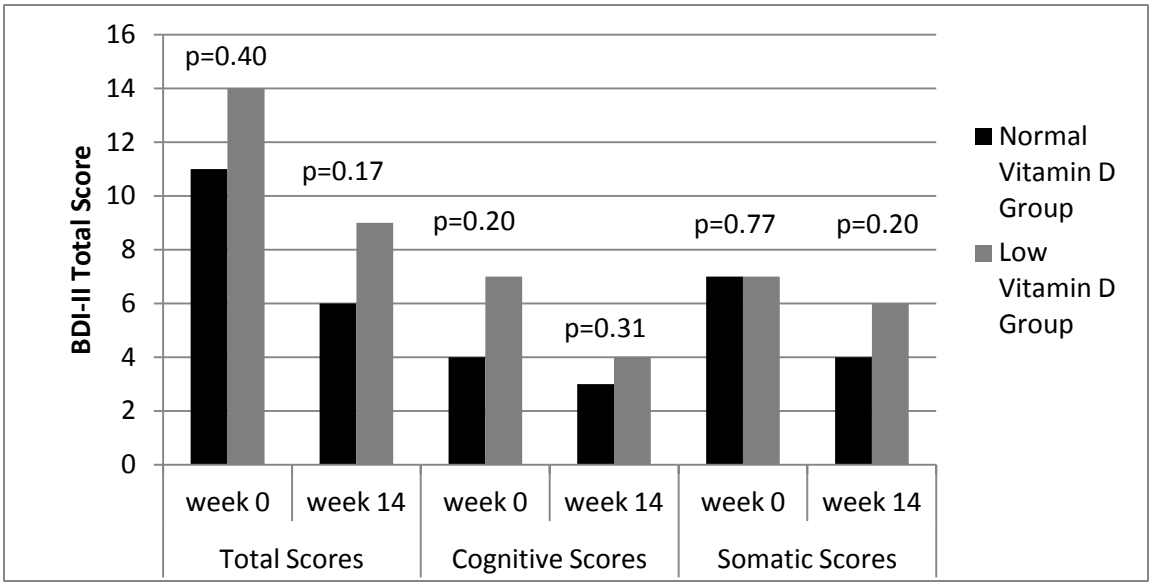
4.8.1.1 Depression scores in patients with IBD (including CD and UC)

There were slightly higher total BDI scores in the low vitamin D group at week 0 compared to the normal vitamin D group (14.0, IQR: 8.0-18.0 vs. 11.0, IQR: 7.0-18.0, respectively, p=0.40). Furthermore, after stratifying the questionnaire into cognitive and somatic categories, there was a trend towards higher cognitive scores at baseline in the low vitamin D group compared to the normal vitamin D group (Figure 4.24 A). Both groups underwent drastic improvement in their total scores from week 0 to week 14, with a similar decrease in both the normal vitamin D and low vitamin D groups (-2.0 IQR: -8.0 – 1.0 vs. -3.0 IQR: -7.0 –0.0, respectively p=0.87).

After stratifying by disease severity, the trends were similar among the patients with severe disease and non-severe disease, with slightly higher depression scores in the low vitamin D group at baseline, once again driven by cognitive symptoms. Interestingly, among patients with non-severe disease, depression scores at week 14 were significantly higher in the low vitamin D group compared to the normal vitamin D group (11.0, IQR: 7.0-22.0 vs. 3.0, IQR: 1.0-5.5, respectively p=0.006), as presented in Figure 4.24 B. After stratifying the questions into cognitive and somatic symptoms in this non-severe group, there was a significantly higher median somatic score at week 14 in low

vitamin D group compared to the normal vitamin D group (7.0, IQR: 4.0 – 9.0 vs. 1.50, IQR: 1.0 – 2.50, respectively p=0.006).

A.



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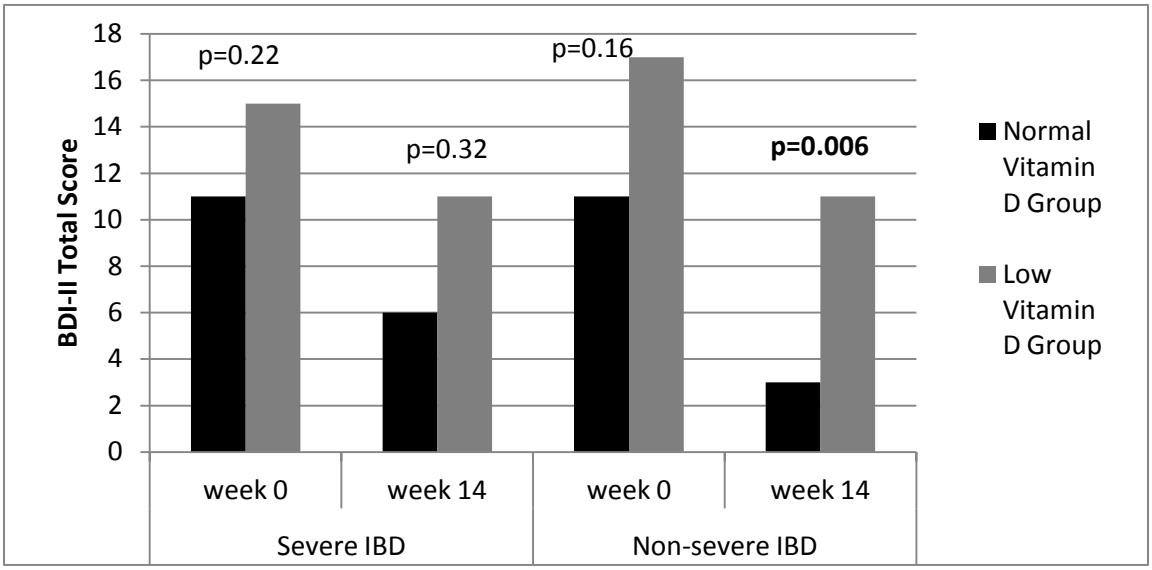

Figure 4-23 Total depression scores at week 0 and week 14 in IBD patients on anti-TNF therapy. A. Total BDI-II scores, cognitive scores, and somatic scores in IBD patients, stratified by vitamin D status. **B.** Total BDI-II scores in IBD patients, stratified by vitamin D status, in the severe-disease group and non-severe disease group.

4.8.2 Week 22 Patient Population

There were 38 patients who completed up to week 22 of the study. There were 9 patients excluded from this analysis, as we did not have completed data on these patients at either week 0 or week 22. Of the 9 patients not included, 5 patients went on to surgery before week 14, 1 patient did not complete the questionnaire, and 3 patients did not answer all of the questions of the BDI-II questionnaire and therefore, their final score could not be calculated. As a result, there were 29 patients with IBD included in this analysis, with 17 patients in the normal vitamin D group and 12 patients in the low vitamin D group. Patients were younger in the low vitamin D group. All other baseline characteristics were similar. The demographics of these patients are presented in Table 4.23 and the distribution of disease severity of patients before initiating anti-TNF therapy is presented in Table 4.24. There was a similar distribution of patients with mild, moderate and severe disease between the two groups.

Table 4-23 Depression at week 22: Demographics of IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Characteristics	Normal Vitamin D Group (n=17)	Low vitamin D Group (n=12)	P-value
Age (years) Median (IQR)	46.0 (32.0-61.0)	30.5 (22.5-37.0)	0.043
Male %, n	35%, 6	67%, 8	0.096
Disease Duration (years) Median (IQR)	5.0 (0.0-14.0)	9.0 (2.0-16.5)	0.590
Crohn's Disease %, n	82%, 14	75%, 9	0.630
Disease Location %, n			
Colonic	18%, 3	25%, 3	0.740
Ileal	35%, 6	42%, 5	
Ileocolonic	29%, 5	8%, 1	
Pancolitis	6%, 1	8%, 1	
Left-sided colitis	12%, 2	17%, 2	
Disease Behavior %, n			
Inflammatory	82%, 14	92%, 11	0.650
Penetrating	12%, 2	8%, 1	
Stricturing	6%, 1	0%, 0	
Infliximab %, n	88%, 15	83%, 19	0.710
Smoking Status %, n			
Current	24%, 4	17%, 2	0.380
Nonsmoker	41%, 7	67%, 8	
Former Smoker	35%, 6	17%, 2	
History of Surgery %, n	24%, 4	25%, 3	1.00
Sulfasalazine/Mesalamine %, n	24%, 4	25%, 3	1.00
Immunosuppressants %, n	71%, 12	92%, 12	0.170
Corticosteroids %, n	41%, 7	50%, 6	0.640

Table 4-24 Depression at week 22: Distribution of disease severity in IBD patients initiating anti-TNF therapy, stratified by vitamin D status

Disease Severity	Week 0		P-value
	Normal Vitamin D Group	Low Vitamin D Group	0.710
% can't assess	18%	8%	
% mild	24%	33%	
% moderate – severe	59%	58%	

4.8.2.1 Depression scores in patients with IBD (including CD and UC)

The low vitamin D group trended towards higher depression scores at baseline (16.5, IQR: 11.75-17.75) compared to the normal vitamin D group (9.0, IQR: 5.5 – 16.5), (p=0.066), which is likely driven by cognitive symptoms (7.50, IQR: 4.5-8.5 vs. 3.0, IQR: 1.0-7.0, p=0.066), as presented in Figure 4-25. By week 14, there was improvement in the median scores, with minimal depression scores in the low vitamin D group (10.0, IQR: 6.0-14.0) and normal group (6.0, IQR: 2.0-8.0), (p=0.066). By week 22, the depression scores continued to be similar between the low vitamin D group and normal vitamin D group (8.5, IQR: 4.5-15.25 vs. 6.0, IQR: 2.0-10.5, p=0.50); however, the decrease in scores from week 14 to week 22 was larger in the low vitamin D group after supplementation compared to the normal vitamin D group (-1.00, IQR: -4.0-1.0 vs. 0.0, IQR: 0.0-3.0, p=0.026).

After stratifying by disease severity, results at week 0 and week 14 were similar to the results of the patients who completed up to week 14. By week 22, depression scores were similar between the low and normal vitamin D groups.

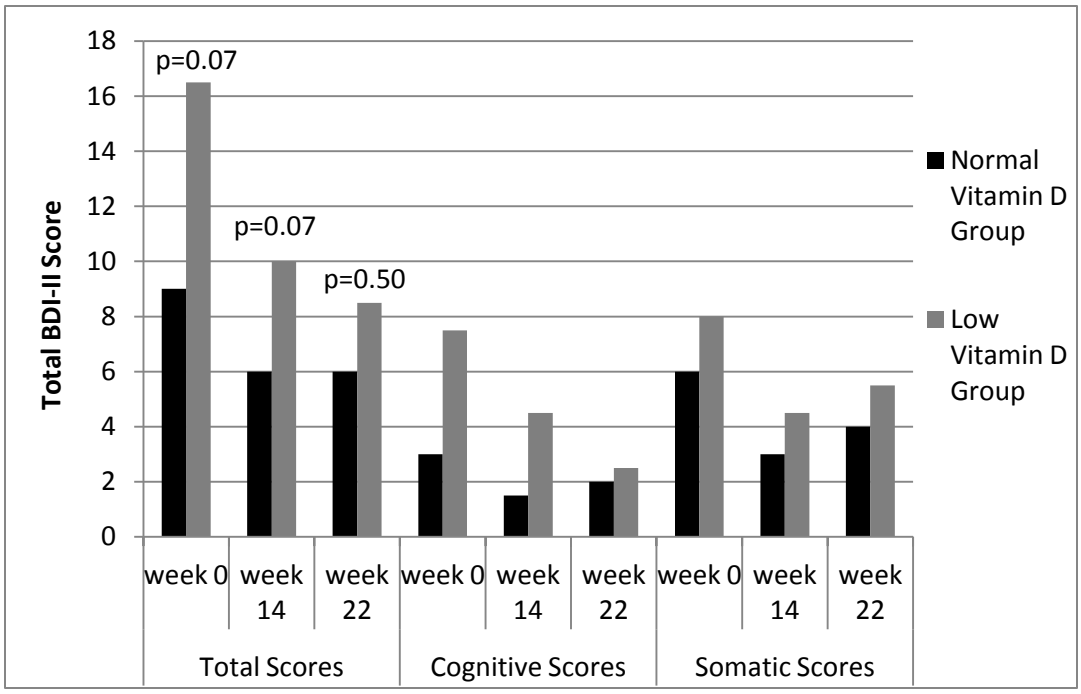

Figure 4-24 Total depression scores, cognitive scores, and somatic scores at week 0, week 14, and week 22 of IBD patients on anti-TNF therapy, stratified by vitamin D status. There was a larger improvement in depression scores from week 14 to week 22 in the low vitamin D group, after vitamin D supplementation, compared to the normal vitamin D group (p=0.026).

5 Discussion

5.1 Overview of Study Purpose

Within the gastrointestinal tract, a unique balance must be maintained between the immune system and gut microflora, wherein the innate and adaptive immune systems respond to mitigate inflammatory signals while tolerating environmental factors including the microflora.[95] This balance can be altered by genetic and environmental factors, and an imbalance can lead to inflammatory bowel disease.[3] As a result, understanding the role environmental factors play in the pathogenesis and progression of this disease will be important in advancing our understanding of the immune defects in IBD and will therefore help improve how we can treat these patients and even potentially lead us to individualized therapy.

Vitamin D is an environmental factor that has been demonstrated to play a role in IBD. It is clear that vitamin D and its receptor impact immune responses, specifically suppressing Th1 driven immune responses.[26,27,37] Cells of the innate and adaptive immune systems constitutively express vitamin D receptors, which has led to the understanding that vitamin D is an immunomodulator of complex immune responses in various autoimmune diseases, such as IBD.[26,96,97]

Vitamin D deficiency is common among patients with IBD.[22] However, it remains unclear whether its deficiency contributes to the pathogenesis of IBD or is a consequence of it. Currently there is no cure for IBD, and the goals of treatment are induction and maintenance of remission.[18] With anti-TNF therapy located at the top of the IBD treatment pyramid, wherein failure is likely to result in surgery[15], it is important to find ways to improve response rates to infliximab and adalimumab.

Studies have shown that vitamin D supplementation has therapeutic benefit in patients with IBD[51,53,54]; however, there are no studies examining the role vitamin D status may

play in the response patients have to IBD-therapies, specifically infliximab and adalimumab. Furthermore, it is also unclear whether supplementing vitamin D deficient patients after anti-TNF-induction therapy has an impact on clinical outcomes.

To review, patients who were initiating anti-TNF therapy were recruited for study participation and followed for 22 weeks. In part 1 (prospective observation), patients were followed for 14 week, wherein we examined the impact of vitamin D status on clinical response rates to induction therapy. In part 2 (vitamin D rescue), patients were followed for an additional 8 weeks, wherein patients with low vitamin D levels were supplemented at week 14, and clinical response was assessed once again at week 22. Assessment of clinical remission, CRP response, cytokine profiles, quality of life, and depression were completed as secondary outcomes.

5.2 Efficacy at week 14: Higher anti-TNF-induced response rates are achieved in IBD patients with severe disease and low vitamin D levels

To date, there are no prospective human studies looking at the effects of vitamin D status on anti-TNF-induced response. We hypothesized that in patients initiating anti-TNF therapy there would be a higher proportion of patients who would clinically respond at week 14 in the normal vitamin D group compared to the low vitamin D group. Interestingly, the proportion of patients who responded in the normal vitamin D group (67%, 14/21) was similar to the low vitamin D group (65%, 15/23), ($p=0.919$).

Large clinical trials that examined anti-TNF-induced response in IBD did not use week 14 or week 22 as study time points; as a result, it is difficult to compare response rates at different weeks. Therefore, for this discussion, we compared response rates to other studies using the proportion of patients who responded to induction therapy and then the proportion who responded to maintenance therapy. As a result, our response rates are similar to previous studies wherein the proportion of patients who clinically

responded to induction anti-TNF therapy have been reported to range from 35%-88%.[12,13,98] Clinical response rates after 4 weeks of infliximab or adalimumab in moderate to severe Crohn's disease patients were 65% and 36%, which is comparable to the rates we demonstrated in this study.[99,100] Although we excluded patients with fistulizing disease from our response analysis, it is interesting to note that the response rate in patients from the ACCENT II trial (A Crohn's disease Clinical trial Evaluating infliximab is effective in treating fistulizing CD) who had fistulizing disease had a similar response rate of 69% (195/282) after infliximab induction therapy (6-12 weeks).[101] Furthermore, similar clinical response rates have been reported for patients with ulcerative colitis, with a rate of 69%[83] at week 8 after infliximab and 55%[102] at week 8 after adalimumab. Additionally, a study with a similar number of patients showed a clinical response rate of 48% (13/27) by week 12 in patients with Crohn's disease after one infliximab infusion.[99] As a result, we have demonstrated similar response rates to previous studies, regardless of vitamin D status.

A difference in the clinical response rates at week 14 was demonstrated between patients with low vitamin D and normal vitamin D after stratifying the patients by disease severity. Interestingly, 79% (11/14) of patients in the low vitamin D group responded by week 14 if they had severe-disease compared to 53% (9/17) of patients who responded in the normal vitamin D group if they had severe disease. This difference did not reach statistical significance (p=0.14); however, a difference of 15-20% is often considered clinically meaningful, suggesting that this difference of 25% in the response rate in our cohort is clinically significant. The opposite trend was demonstrated in patients with non-severe disease, wherein a higher proportion of patients achieved clinical response at week 14 in the normal vitamin D group (100%, 4/4) compared to the low vitamin D group (44%, 4/9), (p=0.11). The patient numbers are small in each group; however, it is evident there are differences in the proportion of patient who achieve a clinical response at week 14 as a consequence of vitamin D status after stratifying by disease severity.

These results were similar in the Crohn's disease population and corresponded with the disease activity questionnaire scores. The low vitamin D group had a lower median HBI score at week 14 compared to the normal vitamin D group, if patients had severe disease, and the low vitamin D group had a higher median HBI score at week 14 compared to the normal vitamin D group, if patients had non-severe disease. All ulcerative colitis patients had severe disease, with higher response rates at week 14 in patients with low vitamin D compared to patients with normal vitamin D.

Clinical trials examining anti-TNF induced clinical response in IBD patients only included patients with moderately to severely active disease, defined by a baseline Crohn's disease Activity Index (CDAI) of 220-450 or a Mayo score of 6 – 12 points (endoscopy score of at least 2)[12,83]; as a result, when comparing our response rates to previous studies, the addition of patients with non-severe disease defined by the Harvey Bradshaw Index questionnaire may have resulted in a different patient population. However, when specifically looking at the patients with severe disease without separating patients by their vitamin D level, their response rate was similar to previous studies at 65% (20/31). As a result, vitamin D status seems to be playing a role in degree of clinical benefit patients receive by week 14 from anti-TNF therapy.

When examining the clinical response rates of those patients who completed up to week 22 of the study, there is a discrepancy in the proportion of patients who responded at week 14 in each vitamin D group compared to those patients who only completed up to week 14. The proportion of patients who responded in the normal and low vitamin D at week 14 (61% (11/18) vs. 79% (11/4), respectively, p=0.29) was more similar to the response rates of the severe-disease patients who just completed up to week 14. By including more patients in the analysis, this difference was washed out. As a result, these patients who completed up to week 22 are more similar to the severe-disease patient population who completed up to week 14. This is most likely due to the fact that the patients removed from the week 22 analysis and who were in the week 14 analysis were patients with non-severe disease. Furthermore, in that there were few

patients who had non-severe disease in this study cohort, the removal IBD patients with non-severe disease in the low vitamin D group changed the patient population to a more severe disease phenotype. There was a similar number of patients removed with severe disease from the low and normal vitamin D groups. Moreover, in patients who completed up to week 22, there continued to be a strong trend towards a higher response proportion in patients with severe disease and low vitamin D levels compared to patients with severe disease and normal vitamin D levels.

Investigation of remission at week 14, a more robust endpoint defined by a HBI score <5 or a PM score of 0 or 1, showed similar and statistically stronger trends. More patients achieved clinical remission after 14 weeks of anti-TNF therapy in patients who initiated this drug with severe disease and low vitamin D levels compared to patients with severe disease and normal vitamin D levels (62% (8/13) vs. 14% (2/14), p=0.018). Additionally, more patients achieved clinical remission at week 14 who started the treatment with non-severe disease and normal vitamin D levels compared to patients with non-severe disease and low vitamin D group (100% (4/4) vs. 29% (2/7), p=0.061). Once again, as a group, the patients with severe disease had a clinical remission rate of 37% (10/27), which is similar to clinical remission rates of 20-40% reported in the literature for both Crohn's disease and ulcerative colitis.[83,99,100,101,102] As a result, identifying a patient's vitamin D status and disease severity before he/she initiates anti-TNF therapy may be important for predicting how likely this patient will respond to therapy and achieve remission. This study suggests that it may be beneficial for patients to initiate anti-TNF therapy with a vitamin D level below 75 nmol/L if they have severe disease or with a vitamin D levels greater than 75 nmol/L for patients with non-severe disease. This is contrary to current literature, which has examined the impact of vitamin D status on the durability of anti-TNF therapy in patients with IBD. In a retrospective study, Zator et al.[55] reported that patients with IBD who are vitamin D insufficient are more likely to stop therapy due to loss of response. Furthermore, Ananthraskana et al.[54] reported that patients with low levels of vitamin D had a significant increased risk for IBD-related

surgery and hospital admissions; however, we have found that patients who initiate anti-TNF therapy with severe disease and low vitamin D levels are 1.5 times more likely to respond at week 14, and patients who respond to anti-TNF therapy are less likely to be hospitalized or undergo surgery.[103]

To support our results, low vitamin D levels may be a marker for the type of inflammatory response that is dominating the patient's disease state, which may be more effectively treated with anti-TNF therapy. This is supported by our cytokine data, wherein patients with low vitamin D levels and severe disease had higher levels of TNF-alpha than patients with normal vitamin D levels and severe disease; as a result, these patients with severe disease who are responding to therapy may have a TNF-alpha-mediated disease, wherein there is more TNF-alpha for infliximab or adalimumab to target and neutralize, resulting in decreased TNF-alpha-mediated inflammation and disease activity. This parallel between higher TNF-alpha levels and higher clinical response/remission was not demonstrated in patients with non-severe disease; however, it may be more difficult to find difference due to small number of patients in this group. There is literature to support the immunomodulatory effects of vitamin D on TNF-alpha secretion. Vitamin D suppresses TNF-alpha production from T cells by inhibiting Th1 cell responses which release INF-gamma, IL-2, and TNF-alpha, as well as inhibits the release of TNF-alpha, IL-1alpha, and IFN-gamma from LPS-stimulated human blood monocytes.[26,27,28,35] Furthermore, *in vitro* studies have shown that vitamin D treatment inhibits the TNF-alpha pathways by reducing colonic mRNA expression of TNF-alpha.[40] Therefore, it is plausible that vitamin D deficiency may result in higher levels of TNF-alpha, as there is less inhibition of this cytokine by vitamin D. As a result, IBD patients with low vitamin D levels have a different cytokine profile than those IBD patients with normal vitamin D levels, if patients have severe disease, and this can impact clinical response to anti-TNF therapy. In support, Parsi et al.[104] have suggested that if TNF-alpha levels are suppressed by certain environmental factors in patients with Crohn's disease, these patients would be expected to have a reduced response to

therapies such as infliximab that target and inhibit this cytokine, which is evident in our study.

There are, however, studies that do not support this association. Martinez-boora et al.[105] found higher levels of serum TNF-alpha to be associated with lack of response to infliximab in Crohn's disease patients with fistulizing disease; however, they also reported that levels of TNF-alpha did not change after infliximab treatment. In contrast to our study, we did not examine response in patients with fistulizing Crohn's disease, and therefore, different disease characteristics may explain these differences. Furthermore, patients with rheumatoid arthritis patients with inactive disease had lower levels of TNF-alpha and responded well to infliximab, wherein patients with active RA and high TNF-alpha levels did not.[106] Louis et al.[107], however, did not find a relationship between infliximab treatment response and serum TNF-alpha levels. As a result, it remains unclear in the literature if TNF-alpha levels could aid in determining clinical response.

The efficacy of infliximab and adalimumab is not restricted to their ability to neutralize TNF-alpha activity. The effects of entanercept, an anti-TNF therapy, is isolated to blocking the TNF-receptor and failed to demonstrate clinical benefit in Crohn's disease.[108] As a result, it may be that vitamin D deficiency supports the other actions of infliximab and adalimumab, such as apoptosis of TNF-alpha expressing target cells.[109] Vitamin D and these anti-TNF drugs work to decrease T cell activation and proliferation and stimulate regulatory T cells activity.[5,22] Vitamin D *in vitro* reduces proliferation of T lymphocytes in Crohn's disease patients[110], and infliximab induces T lymphocyte apoptosis in Crohn's disease patients.[111] As a result, it may be hypothesized that there is more T cell proliferation in patients with severe-IBD and low vitamin D levels, and in that these cells express a significant number of transmembrane TNF-alpha,[112] anti-TNF therapy is effective in this subset of IBD patients by binding to membrane-associated TNF-alpha, inducing apoptosis, and in so doing, reducing mucosal inflammation.[109,111,112]

All patients with ulcerative colitis had severe disease before initiating anti-TNF therapy; as a result, the non-severe disease patients had Crohn's disease. It remains unclear as to why there are opposite trends with respect to vitamin D status and clinical response between Crohn's disease patients with severe and non-severe disease. There were a small number of patients in this group; as a result, a larger sample size would aid in understanding this relationship.

C-reactive protein (CRP) is produced by hepatocytes as a part of the non-specific acute-phase response to most forms of inflammation, infection, and tissue damage.[85] It is a biomarker used in IBD as an objective measure of assessment of disease activity and severity, as well as a useful measurement for monitoring response to treatment of inflammation and infection.[85] Symptoms are often subjectively measured by the Harvey Bradshaw Index questionnaire and Partial Mayo questionnaire; however, in combination, laboratory indices and disease activity questionnaires can be used to assess severity and are less invasive than endoscopy.[86]

Studies have demonstrated that higher CRP before initiation of anti-TNF therapy predicts a better response to therapy, with a median CRP before treatment of 16.8 mg/L in responders compared to 9.6 mg/L in nonresponders, p=0.02.[113] As a result, raised CRP may select patients with active gut inflammation who are more likely to clinically respond; however, CRP levels at baseline in our cohort were similar between patients with low and normal vitamin D levels. Furthermore, median CRP levels in both vitamin D groups were high (>20 mg/L), indicating inflammation before treatment initiation.

The proportion of patients who achieved a CRP response at week 14 was similar between the groups, even after stratifying by disease severity and IBD type. Interestingly, the proportion of patients with Crohn's disease who achieved CRP normalization at week 14 were slightly different between the vitamin D groups, with more patients achieving a CRP level <8 mg/L in the normal vitamin D group compared to the low vitamin D group (83% (10/12) vs. 67% (12/18), p=0.312); this difference

increased in Crohn's disease patients with severe disease (80% (4/5) vs. 56% (5/9), p=0.58). Furthermore, when examining CRP normalization at week 14 in IBD patients who completed up to week 22, there continued to be a trend towards more patients achieving a normal CRP by week 14, if they had normal vitamin D levels. As discussed previously, patients who completed up to week 22 are more similar to the severe-Crohn's disease population who completed up to week 14. As a result, achieving CRP normalization may be influenced by vitamin D status in patients with severe-Crohn's disease. The strong CRP response in the Crohn's disease patients is supported by previous data wherein a strong CRP response had been observed in Crohn's disease with only a modest CRP response in patients with ulcerative colitis.[114] However, the severe-Crohn's disease patients with low vitamin D levels were responding better clinically, using a subjective measure, but were not responding as well on an objective measure. CRP is a good marker of disease activity[114]; therefore, it is conflicting to see that these patients may be experiencing more inflammation but subjectively feeling well and more likely in clinical remission than patients with normal vitamin D levels.

The main stimulus for CRP production is IL-6 and this response is enhanced in combination with IL-1beta and TNF-alpha[113]. The levels of these cytokines were higher in the Crohn's disease patients with severe disease in the low vitamin D group at baseline, which would suggest there is more inflammation in the severe-Crohn's disease patients with low vitamin D levels compared to those patients with normal vitamin D levels. Subsequently, this may explain why fewer patients achieved normal CRP levels by week 14, if they had higher inflammation before drug initiation. We would then also expect that these patients would have higher CRP levels at baseline; however, median CRP levels were similar between the low and normal groups at baseline (22.0 mg/L IQR: 12.4 – 59 vs. 12.1 mg/L IQR: 10.5 – 27.3, respectively p=0.298) and week 14 (5.2 mg/L IQR: 3.4 – 12.6 vs. 6.1 mg/L IQR: 3.8 – 6.6, p=1.0). Furthermore, by week 14, these three cytokine levels were similar between the groups and not likely to explain why the rate of CRP normalization in the low vitamin D group was slightly lower. The delta change in

CRP from baseline to week 14 is similar between the groups; as a result, in that the number of patients is very small, these differences are not statistically significant, and a difference of one person greatly changes the percentage outcome in a small sample size. Therefore, this is not clinically significant. It would be interesting, however, to see if this trend continued in a larger sample size. Overall, CRP, as a marker of inflammatory activity, was high in both groups at baseline even after stratifying by disease severity and disease type, and by week 14 the degree of inflammation improved within both groups. Consequently, CRP normalization was not indicative of clinical response to anti-TNF therapy.

This study demonstrated that a high proportion of patients achieved a CRP response and normalization in both groups regardless of vitamin D status after 14 weeks of infliximab. Furthermore, Jurgens et al.[114] showed CRP normalization in 61% of Crohn's disease patients after induction infliximab therapy, which is similar to the rates we demonstrated in this cohort. Additionally, they reported that almost 45% of the patients who achieved primary clinical response showed early CRP normalization; as a result, less than 50% of patients who clinically respond will achieve a normal CRP by week 14. Anti-TNF therapy affects the underlying pathology of CRP production, and in that TNF-alpha drives CRP, the patients had improved CRP levels as a result of anti-TNF therapy. Therefore, CRP does not correspond with vitamin D levels in predicting response.

5.3 Efficacy at week 22: Higher anti-TNF induced response rates are maintained in IBD patients with severe disease and low vitamin D levels

Vitamin D supplementation in patients with low vitamin D levels had no effect on the clinical response patients had by week 22. The proportion of patients in the low vitamin D group who responded at week 22 remained the same, with a 25% higher response rate compared to patients in the normal vitamin D group. This also occurred among patients with severe disease, wherein the proportion of patients who responded in the

low vitamin D group with severe disease at week 22 was significantly larger than in the normal vitamin D group. Studies have shown anti-TNF therapy to be effective in treating patients with IBD with overall induction response rates of up to 88%[98], and patients with low vitamin D had a response rate at week 14 of 79% and of 89% in those with severe disease; therefore, additional response was very unlikely. Interestingly, not all patients maintained a response by week 22 in the normal vitamin D group. Clinical response at week 14 was 61% and 50% at week 22. This was also demonstrated in patients with severe disease (50% at week 14 vs. 43% at week 22) and in patients with non-severe disease (100% at week 14 vs. 75% at week 22). Studies have shown that between 50-60% of IBD patients treated with infliximab or adalimumab eventually lose response, with a median time to lose of response ranging from 28 weeks to 100 weeks, depending on the type of anti-TNF therapy and type of IBD.[115,116] As a result, it is common for patients to lose response. Interestingly, this decrease in the proportion of patients achieving a clinical response parallels the increase in CRP seen in the normal vitamin D from week 14 to week 22, specifically in patients with severe disease. The proportion of patients with normal vitamin D and severe disease who achieved CRP normalization decreased from week 14 to week 22 (100% (4/4) vs. 20% (1/5), p=1.0). It is not statistically significant, which is likely due to a small sample size; however, the delta change in the median CRP levels from week 14 to week 22 is different between the normal vitamin D group and low vitamin D group (5.40, IQR: 1.35-7.80 vs. -0.60, IQR: -7.90-1.05, respectively p=0.048), wherein the median CRP level in normal vitamin D, severe disease group by week 22 was above 8 mg/L and larger than the median CRP level in the low vitamin D group with severe disease. The increase in CRP in the normal vitamin D group may be an indication that these patients are losing response, wherein increases in CRP have been reported to precede clinical relapse in 70% of IBD patients on maintenance infliximab; as a result, it would be interesting to have followed these patients after the increase in CRP and examined their outcomes. Overall, IBD patients with severe disease and low vitamin D levels had a strong clinical response to anti-TNF induction therapy and maintained this response in parallel with a higher proportion of

patients who achieved CRP normalization at week 22. IBD patients with severe disease and normal vitamin D levels had a weak response to anti-TNF induction therapy, regardless of a high proportion of patients who achieved normal CRP levels, and by week 22, seem to have begun to lose response to therapy in parallel to increasing CRP levels above the normal range.

Vitamin D supplementation may have a played a role in lowering CRP levels; however, our cytokine data does not support this. As a result, it likely that patients were responding to the drug and the additional anti-TNF dose after the induction phase improved CRP levels by week 22. In that the normal vitamin D group did not improve after the additional dose of infliximab after the induction phase, patients may not have been responding the induction doses and therefore, an additional dose did not make a difference in improving their disease activity. It would be interesting to see if these patients were dose escalated after their 5^{th} infusion.

The fact that a sub-group of patients are responding to the drug, but their CRP levels are slightly higher may be because of additional inflammation that is not interfering with their clinical response or from an infection or other factors that were not controlled for. There were still a high proportion of patients who achieved CRP normalization at week 14 in the low vitamin D groups and in the end it may not be different from those patients who had normal vitamin D levels. By week 22, in patients with severe disease, CRP levels between the normal vitamin D group and low vitamin D group after supplementation were significantly different, with lower CRP levels in the low vitamin D group after supplementation. CRP response and normalization rates in the normal vitamin D group dropped from week 14 to week 22, while these rates increased in the low vitamin D group after supplementation. This additional CRP response is likely explained by the additional dose of anti-TNF therapy.

5.4 Cytokine Responses: TNF-alpha, IL-6, and IL-1beta are higher in IBD patients with severe disease and low vitamin D levels before initiating anti-TNF therapy

The secretion of TNF-alpha, IL-6, and IL-1beta have been documented to be released simultaneously from lamina propria mononuclear cells of IBD patients and are important in the initiation and perpetuation of chronic inflammatory responses in IBD.[117] There is evidence to support that these cytokines are further elevated under low vitamin D conditions. VDR/IL-10 double KO mice express two-three fold higher levels of IL-1beta, IL-2, IFN-gamma, and TNF-alpha mRNA in their colons than single KO mice.[37] Furthermore, even healthy individuals had higher levels of LPS-induced TNF-alpha, IL-6, IL-1beta, and IFN-gamma in the winter months compared to the summer months.[118] As a result, vitamin D and its receptor regulate these cytokines and low vitamin D levels may explain the higher levels seen in these patients. Interestingly, in this study, the effects of vitamin D status on cytokine responses were only demonstrated in patients with severe disease. As a result, it may be that vitamin D deficiency enhances inflammatory responses occurring in patients with severe disease. In other words, the inflammatory responses are exacerbated in IBD patients with low vitamin D levels because there is not enough vitamin D substrate to reduce and regulate inflammatory responses.

By week 14, the significant decrease in IL-6 and TNF-alpha levels from baseline in the severe-low vitamin D group was likely a result of anti-TNF therapy, and is likely to play a role in the high clinical response achieved in this group after induction therapy. TNF-alpha and IL-6 work synergistically in preventing intestinal T-cell apoptosis, and anti-TNF treatment decreases IL-6 levels in addition to TNF-alpha levels.[119]

It is, however, unknown how vitamin D supplementation will impact future clinical outcomes in these patients who have responded very well to anti-TNF therapy by week 14. It may be that since patients have achieved a good response to therapy and pro-

inflammatory cytokines have been suppressed due to the drug, that vitamin D supplementation may be supportive in maintaining this response by synergistically working with the anti-TNF drugs to suppress of TNF-alpha and Th1 immune responses. However, it could be hypothesized that vitamin D supplementation is detrimental to these patients as this TNF-alpha mediated disease as a consequence of low vitamin D levels is important for this drug to continue working and acting on neutralizing this cytokine and suppressing Th1 responses. The serum TNF-alpha levels at week 14 were similar within the two vitamin D groups with severe disease which suggests that vitamin D status is not a factor in regulating TNF-alpha levels after anti-TNF induction therapy. Similar TNF-alpha levels were demonstrated at week 22 as well. We, however, do not have data on mucosal cytokine levels, which would better reflect the type of intestinal inflammation driving the disease of these patients.

IL-8 is an innate cytokine produced by macrophages and epithelial cells[120], and by week 22, there was a drop in this cytokine in the low vitamin D group following supplementation. The decrease in IL-8 in the vitamin D low group after vitamin D supplementation demonstrates that vitamin D is working to suppress the innate immune system. The immune system is very complex, and there is strong evidence to support the role of vitamin D as an immunomodulator rather than an immunosuppressor. It has been shown to suppress the inflammatory responses of the adaptive immune system while stimulating antibacterial activity of the innate immune system.[22] Its role in regulating IL-8 is not well described; however, Eleftheriadis et al.[121] demonstrated that treatment with paricalcitol, a vitamin D analogue, reduced basal and LPS-induced IL-8 levels from human peripheral blood mononuclear cells. IL-8 is a neutrophil chemoattractant produced at the onset of bacterial infection and initiates recruitment of neutrophils, which destroy pathogens and induces infiltration of T lymphocytes into inflamed tissue.[122] IBD is a neutrophil-mediated disease in which IL-8 levels are elevated to stimulate infiltration of neutrophils in lesions of active Crohn's disease and ulcerative colitis. Furthermore, Mitsuyama et al.[123] found a significant

correlation between intestinal tissue levels of IL-8 and IL-1beta and TNF-alpha; as a result, the reduction in this cytokine is important for these patients as disease activity improves.

It would, however, be interesting to assess the long-term outcomes of vitamin D supplementation, because in a low vitamin D state, these patients achieved great clinical benefit after anti-TNF therapy. Overall, IL-8 can be a signal of inflammation and should decrease as disease improves. Vitamin D treatment has exhibited a decreased IL-8 response; however, in that patients with low vitamin D and severe disease responded very well to therapy and maintained this improvement, with a high proportion of patients achieving normal CRP levels by week 22, a decrease in IL-8 may be reflective of improved disease activity seen in these patients. The normal vitamin D group with severe disease demonstrated increasing CRP and loss of response; as a result, it is difficult to compare the effects of vitamin D supplementation between these two groups had different degrees of disease activity. Further analysis is needed to examine patients who had similar disease activity at week 14 and week 22 in both groups, such as those who responded at week 14 and maintained response at week 22 in both groups to determine the effects of vitamin D supplementation on cytokine responses. This would reduce the confounding effects of disease activity on the cytokine responses after vitamin D supplementation.

5.5 Quality of life of patients on anti-TNF therapy was associated with disease activity, not vitamin D

Despite differences in vitamin D levels, quality of life, measured by the short IBD questionnaire (SIBDQ), was similar in IBD patients before starting anti-TNF therapy and after 14 weeks of anti-TNF therapy. However, after stratifying by disease severity, patients in the severe-low vitamin D group showed greater improvement in quality of life scores from week 0 to week 14 compared to the severe-normal vitamin D group. This is likely due to the greater response to anti-TNF therapy in this group,

demonstrated by decreases in disease symptoms measured by the Harvey Bradshaw Index questionnaire and Partial Mayo questionnaire. Furthermore, after vitamin D supplementation, the low vitamin D group showed greater improvement in quality of life scores compared to the non-supplemented normal vitamin D group. It is unlikely that this improvement in scores was due to the vitamin D supplementation. It is most likely due to disease improvement as a result of another dose of anti-TNF after a good response to induction therapy; patients in the normal vitamin D group did not clinically respond as well at week 14 or week 22. This is supported by reports that anti-TNF therapy improves quality of life as a result of better disease control.[124]

Interestingly, quality of life scores continued to be associated with response rates after stratifying by disease severity. Patients with severe disease in the low vitamin D group had a better clinical response at week 14 and week 22 and there was a trend to higher quality of life scores in this group at week 22. Quality of life scores significantly improved after vitamin D supplementation compared to the non-supplemented normal vitamin D group, if patients had severe disease. This is likely due to continued improvement in disease activity after an additional dose of anti-TNF. Patients in the normal vitamin D group did not respond as well by week 14 or week 22. Patients with low vitamin D and non-severe disease showed the opposite trend, wherein they did not respond as well to anti-TNF therapy at week 14 or week 22. By week 22, quality of life scores were lower than the scores in the non-severe-normal vitamin D group.

Quality of life scores improved as disease activity improved, demonstrating that health status is influenced by disease activity. The SIBDQ is a valid measure of quality of life, but it is also open to clinically important changes in disease activity.[125]

5.6 IBD patients with low vitamin D levels have more depressive symptoms at initiation of anti-TNF therapy

In patients who completed up to week 14, baseline depression scores were not statistically different; however, patients in the low vitamin D group scored in the mild depression range (14-19) while patients in the normal vitamin D group scored in the minimal depression range (0-13) when assessed by the self-administered Beck Depression Inventory-II questionnaire. In that these two groups had a similar distribution of patients with mild, moderate, and severe disease activity at baseline, this study shows that normal vitamin D levels may have a role in minimizing depressive symptoms in patients who have active disease and are starting anti-TNF therapy. Depression and anxiety levels of IBD patients have been shown to be higher than the general population[126] and are increased during periods of activity disease.[59] Additionally, vitamin D may be important in mood disorders and brain function, as it has been shown to act as a neurosteroid. Animal studies have demonstrated that vitamin D plays a role in the expression of monoamines, such as norepinephrine, serotonin, and dopamine, which are involved in depression.[127] It is interesting in that after stratifying the questions into cognitive or somatic subscores, the depression scores in the low vitamin D group were driven by cognitive symptoms. These include symptoms such as sadness, pessimism, past failure, loss of pleasure, guilty feelings, self-dislike, self-criticalness, suicidal ideation, crying, agitation, loss of interest, indecisiveness, and worthlessness.[128] Somatic symptoms include loss of energy, sleep problems, irritability, appetite problems, concentration, fatigue, and loss of interest in sex.[128] Previous studies have shown that in patients with Crohn's disease, active disease is associated with fatigue, depression, and sleep disturbance[129]; as a result, the somatic symptoms are likely driven by the patients' disease, which were similar between the two groups at baseline. Depressive symptoms did improve in both groups from baseline to week 14, signifying that these patients were feeling better on anti-TNF therapy, and both groups reached a median score in the range of minimal symptoms by week 14.

In that there were differences in the drug-response rates after stratifying by disease severity, depression was also examined between the two vitamin D groups in patients with severe disease and patients with non-severe disease. Despite disease severity, patients with low vitamin D levels continued to have a median BDI-II score in the mild depression range while patients with normal vitamin D levels had a median baseline score in the minimal depression range. Additionally, by week 14, depressive symptoms improved, wherein both groups had a median score of minimal depression. It is of interest, however, that patients with non-severe disease in the low vitamin D group had significantly higher depression scores at week 14 compared to patients in the non-severe-normal vitamin D group (11.0, IQR: 7.0-22.0 vs. 3.0, IQR: 1.0-5.5, p=0.006), and this was heavily driven by somatic symptoms. Previous studies have used BDI-II scores <4 as a cut-off for very low symptoms,[130] and the non-severe-normal vitamin D group fell within this range. When comparing this result to the drug-induced clinical response rates, it is likely that the higher somatic scores in the non-severe, low vitamin D IBD patients may be a consequence of the lower response rates seen in this group; these patients were not achieving the same clinical benefit from the drug as the normal, non-severe IBD patients and continued to have disease activity. Symptoms like loss in energy and fatigue may be explained by an inflammatory state.

Overall, low vitamin D levels seem to be associated with more depressive symptoms, specifically of the cognitive subtype; however, over time, these symptoms improve with anti-TNF therapy. In patients with non-severe disease, higher symptoms of depression at week 14, driven by somatic symptoms, are likely due to the lack of response these patients are having to anti-TNF therapy.

Similar baseline and week 14 depression scores were reported in those patients who completed up to week 22 of the study. Overall, depression scores remained relatively stable in patients with normal vitamin D levels at week 0, week 14, and week 22. At each of these time points, scores remained in the range of minimal depression. There was a trend to higher depression scores in the low vitamin D group at baseline, as these

patients scored in the mild depression range; however, by week 14, these scores improved into the mild range and continued to remain stable by week 22. As a result, vitamin D supplementation did not improve depressive symptoms. Scores slightly improved from week 14 to week 22 in the severe-low vitamin D group after vitamin D supplementation; however, this is likely due to continued improvement in the patients' disease activity as a result of a good response to anti-TNF therapy.

The use of self-reported questionnaires rather than clinical diagnoses to assess symptoms of depression, anxiety, and stress is a limitation. While self-report assessment has been established as a valid means for assessing mental health difficulties,[131,132] there is a risk of under and over reporting. Our results suggest an association between baseline serum 25(OH)D concentrations and self-reported depressive symptoms, rather than an association with a clinical diagnosis of depression. Furthermore, we did not control for anti-depression medication or inquire about other mental health issues such as anxiety. Corticosteroids are used to induce remission in patients with active disease and are known to cause depressive symptoms.[59] However, depression associated with corticosteroids plays a role in the overall disease process and therefore is not considered a confounder in this study. Furthermore, in that similar rates of depression between patients with Crohn's disease and ulcerative colitis have been reported[133] and depression was assessed in very few UC patients, depression was not analyzed separately for the two diseases.

5.7 Study Limitations

The primary limitation of our study is the absence of the CDAI and total Mayo scores to determine clinical response and remission. This limits the direct comparison with remission and response rates calculated in randomized clinical trials; however, improvement in general well being, abdominal pain, diarrhea, and extra intestinal symptoms has been demonstrated to closely correlate with the calculated CDAI. Additionally, the partial Mayo score is also closely correlated with the total Mayo score.

Furthermore, the frequency of diarrhea within the HBI may introduce the possibility of single-parameter bias.[134] In order to limit this bias we defined a clinical response as a decrease in the HBI scores of at least 3 points, wherein other studies have used a decrease of 2 points. Parsi et al.[104] used the same decrease of 3 points in the HBI score to define a clinical response in their Crohn's disease patients; however, they also gave a maximum of 1 point for the reduction of liquid bowel movements. This would stabilize clinical response results among patients with HBI scores that are driven by bowel movements, and may be useful for future studies. Other limitations in regards to the HBI questionnaire would be inclusion of patients with ostomies and multiple surgeries; however, the bowel movements in these patients were no different than the other patients. Moreover, the use of pain medication such as codeine and/or anti-diarrheal medication was not controlled for and could have influenced the number of bowel movements patients were having at each time point.

Treatment decisions are not solely based on clinical indices or solely based on inflammatory biomarkers such CRP; they are best used in combination. Bjorkesten et al.[135] reported that clinical disease activity assessments or CRP were not capable of reliably discriminating remission from active disease and combining the use of HBI scores and CRP was more effective in identifying endoscopic remission compared with either test alone. For this study, CRP, collected from standard of care blood work, and clinical response, defined by clinical disease activity scores, were assessed separately. Studies that have examined response to anti-TNF therapy have used a physician's global assessment to determine response and remission in patients to this treatment. Teshima et al.[98] examined efficacy of infliximab in the same centre as this study and reported a higher clinical response rate to induction therapy of 88% (117/133), using a physician's global assessment at 10 to 12 weeks after the first infliximab infusion; as a result, using individual CRP levels and clinical index scores together would have been a stronger way to determine if a patient was responding or in remission.

Adding fecal calprotectin would have added strength in determining disease activity at each of the time points; however, patient recruitment was difficult due to the small number of patients starting anti-TNF therapy and asking patients for stool samples may have decreased the number of patients recruited. Fecal calprotectin, a neutrophil-derived protein has been shown to correlate with both endoscopy and histological findings in luminal Crohn's disease and a normal fecal calprotectin concentration is a reliable surrogate marker for endoscopic inactive disease.[135] Bjorkesten et al.[135] demonstrated that only fecal calprotectin is a reliable noninvasive marker for endoscopic remission in clinical practice, and combining it with clinical indices could be more specific and sensitive. Moreover, a proportion of patients who have low CRP values still report symptoms of active disease, which may be due to other factors such as irritable bowel syndrome. As a result, patients who had an increase in their clinical disease activity score may be dose escalated by their gastroenterologist; consequently, patients may receive escalation of infliximab therapy for symptoms that are unrelated to their disease activity, and this may have resulted in an overestimation of non-response in our cohort. As a result, using fecal calprotectin would have been effective in distinguish coexisting irritable bowel syndrome-like symptoms from occult inflammation.[136]

Another limitation is that CRP is nonspecific to gut inflammation and can be produced by viral or bacterial stimuli; as a result, studies reporting the correlation between CRP and clinical indices are inconsistent.[135] In this study, patients were not assessed for other factors that may have influenced CRP levels at week 14 or week 22. For example, *Clostridium difficile* infection is common among patients with IBD[137] and if a patient was infected with this bacteria at any of these time point, his/her CRP would have increased or been high and not reflective of their disease state.

The timing of clinical response assessment was variable and therefore a limitation of this study. Infliximab induction includes a dose at week 0, week 2, and week 6, and for this study, clinical response to induction therapy was recorded at their 4th dose, which

should be at week 14. Clinical response was then once again assessed at the patient's 5th dose, which should be at week 22. However, patients' lifestyles are variable and not all patients were administered their 4th dose at week 14 and 5th dose at week 22. As a result, clinical disease activity questionnaires were completed and blood work was collected at each patient's 4th and 5th infliximab infusion. Further analysis is required to determine how variable the interval between the 3rd and 4th dose and 4th and 5th dose was between the two groups. If there were more patients in the normal vitamin D group who had a longer time to their 4th or 5th dose than patients in the low vitamin D group, this would have confounded our clinical response data as a longer time to infusions may result in under dosing these patients and increasing their likelihood losing response.

Anti-TNF induced response was defined at week 14, which is specific for infliximab induction therapy, but not for adalimumab induction therapy. Technically, clinical response to adalimumab induction should be assessed at week 4. We do have clinical disease activity scores for these patients at this time point; however, since there were very few patients on adalimumab, we grouped these patients with the patients on infliximab. As a result, by week 14, patients on adalimumab would be on maintenance therapy. Therefore, with a larger patient population, it would be best to assess infliximab induced clinical response separately from adalimumab induced clinical response.

Additional limitations include insufficient power to determine differences in response rates between patients with normal vitamin D levels and patients with low vitamin D levels. A sample size calculation determined that approximately 300 patients would be required in each group to detect a difference of 10%, with a power of 80% and a two-sided significance level of 5%, and we could not reach this number by recruiting out of a single IBD centre. Furthermore, when we stratified the groups into patients with Crohn's disease and patients with ulcerative colitis, the sample size became very small (<10). Consequently, the statistical analyses used on these small groups are at risk of Type II error, wherein the probability of obtaining false negatives is increased. As a result,

larger studies with longer follow up periods will be required to validate these results and determine the long-term outcomes of these patients.

Crohn's disease and ulcerative colitis have been demonstrated to have distinct cytokine profiles; as a result, combining patients with Crohn's disease or ulcerative colitis into one group to increase the sample size for certain analyses is a limitation. Crohn's disease is characterized by a Th1 type immune response and ulcerative colitis is characterized by a Th2 immune response; however, these patients were grouped together for cytokine analysis. As a result, assessing these two diseases separately would have removed certain assumptions that may have resulted in a bias that created differences between the expected and true values.

Cytokine levels were measured in serum as a non-invasive method for assessing inflammation and disease activity; however, it is another limitation as this method does not directly assess the type and extent of cytokine responses occurring at the site of the inflamed mucosa. Measuring gene expression of several inflammatory signaling molecules from intestinal tissue would have been a more direct and accurate way of monitoring the type of cytokine responses occurring in our study population.[138] The examination of tissue samples was not feasible for this study; however, it should be noted that there are possible differences between serum and tissue analysis of cytokine levels.

The nutritional status of each participant was not formally assessed. We did assess albumin and it did seem to be lower in patients with low vitamin D levels. Patients with low vitamin D levels did have more systemic inflammation determined by higher levels of specific cytokines compared to patients with normal vitamin D levels, and albumin and 25(OH)D levels have been reported to be reduced during systemic inflammation.[139] These IBD patients with low serum vitamin D also seemed to have lower calcium, hemoglobin and higher white blood cell count and platelet levels. As a result, patients in the normal vitamin D group may be a different population than the patients in the low

vitamin D group and therefore, this may have confounded our results. Weight and height would have also been important factors in the assessment of overall health status. Patients who are underweight for their height and age are likely malnourished and have low fat stores, which is an important reservoir for vitamin D.[69] Additional factors that may have been important in assessing nutritional status would have been osteoporosis and sudden weight loss due to severe disease. Determining the nutritional status of these patients before initiating therapy and over the study period may have been important in assessing the health status of these patients as a whole to determine differences in nutritional deficiencies and therefore determining specific confounding factors that could have been controlled for.

Patients included in each analysis were slightly different, and therefore, assessing results as a whole picture is a limitation. Patients included in the response results were only of inflammatory disease and patients of all disease behavior were included in the cytokine analysis; as a result, we are making the assumption that these cytokine results would be the same if only patients with inflammatory disease were included. Therefore, caution should be taken when we use our cytokine results to help explain the differences seen in the response rates between the low and normal vitamin D groups.

Lastly, it was difficult to control for additional vitamin D supplementation, diet, travel to locations close to the equator, and tanning bed use. Patients were asked at the beginning of the study about the amount of vitamin D they were taking orally as part of the inclusion criteria. However, in that patients with low vitamin D levels at baseline continued to have low vitamin D levels at week 14, it is likely that if they increased the amount of vitamin D during this time period, it did not increase their vitamin D level enough to reach above the normal vitamin D level cut-off.

5.8 Summary of Objectives and Findings

In summary, this study demonstrated that IBD patients with severe disease had a strong clinical response to anti-TNF induction therapy if they had low vitamin D levels before

initiating treatment. This is likely explained by a specific inflammatory response that is 1) dominating the disease state of these patients as a consequence of inadequate immune regulation due to low levels of vitamin D and 2) is effectively treated by the mechanisms of action of infliximab and adalimumab. Furthermore, vitamin D supplementation in these patients after week 14 did not impact clinical response at week 22. Clinical response was maintained over the next 8 weeks, with decreased levels of IL-8 after vitamin D supplementation; this was likely due to continual improvement in disease activity after an additional dose of anti-TNF therapy. On the contrary, IBD patients with severe disease and normal vitamin D levels had a weaker response to anti-TNF therapy induction therapy. By week 22, some of these patients began to lose response to treatment in parallel with increasing CRP levels.

Quality of life did not seem to be influenced by vitamin D status; however, improved quality of life paralleled improved disease activity and clinical benefit as a result of a good response to anti-TNF therapy. In regards to depression, low vitamin D levels were associated with more cognitive depressive symptoms in patients with IBD prior to starting anti-TNF therapy, regardless of disease severity. These symptoms, however, improved by week 14 in both patients with low vitamin D levels and normal vitamin D levels in parallel with improved disease activity.

This is the first study to determine the impact of vitamin D status on anti-TNF-induced response in IBD patients with severe disease. Future studies will be important for examining the long-term outcomes of these patients. Moreover, investigation of vitamin D supplementation in IBD patients with low vitamin D levels just prior to initiation of anti-TNF therapy would improve our understanding of the impact vitamin D has on the inflammatory response dominating these patients' disease and how this would influence clinical response. This knowledge would help us answer the question of whether vitamin D status is a marker of the type of inflammatory responses that are occurring in these patients, and if vitamin D supplementation just prior to the start of therapy would affect clinical response rates at week 14. As a result, the next step will be

to conduct a randomized controlled trial comparing clinical response rates in IBD patients with severe disease and low vitamin D levels who are randomized to receiving vitamin D supplementation or placebo prior to anti-TNF therapy.

5.9 Conclusion

Infliximab and adalimumab are expensive and potentially harmful therapies; as a result, identifying predictors of response will be important advancements in the clinical care of these patients. This will aid in selecting patients who will benefit from this therapy and lead to investigation of other treatment options for those patients who will not benefit. The results of this pilot study are novel and unexpected and will spark further exploration into this relationship between vitamin D and response to anti-TNF therapy. Stronger evidence is required, but vitamin D status may be a strong predictor of anti-TNF induced response in IBD patients with severe disease.

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Appendices

APPENDIX A. Participant Information Sheet

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PARTICIPANT INFORMATION SHEET AND CONSENT

Title of Research Study:

The Role of Vitamin D in Anti-TNF Induced Remission in Patients with Inflammatory Bowel Disease

Principal Investigator: Dr. Karen Kroeker, MD **Sub-Investigator:** Dr. Richard Fedorak, MD

Study Coordinator: Krista Reich

BACKGROUND AND PURPOSE

Vitamin D deficiency is common in patients with inflammatory bowel disease (IBD). The primary role of vitamin D is related to bone health; however, vitamin D has been shown to have a supporting role in regulating inflammation in Crohn's disease and ulcerative colitis.

You are being asked to participate in a research study in order to determine the differences, if any, in the level of clinical improvement between vitamin D deficient and sufficient IBD patients who are being prescribed anti-TNF therapy. We are interested to see if vitamin D levels in IBD patients have an effect on whether or not these patients have clinical improvement in response to anti-TNF therapy.

You are being asked to participate in this research study because:

a) you are 18 years of age or older with a diagnosis of Crohn's disease or ulcerative colitis
b) you are being prescribed anti-TNF therapy (infliximab or adalimumab)

This document describes the study. Feel free to ask the Study Doctor or the Study Staff to explain any words or information that you do not clearly understand.

PROCEDURES

If you agree to participate in this study, you will have 3 visits over 22 weeks if you are starting anti-TNF therapy or 2 visits in 8 weeks if you are on maintenance therapy. Each visit will take approximately 20-30 minutes.

Part 1: Anti-TNF New Starts

Week 0/Baseline:

- Complete 2 questionnaires related to quality of life and a demographic information sheet (10-15 minutes)
- Collection of a blood sample to measure vitamin D levels & identify specific biological markers
- Collection of a urine sample
- Completion of a questionnaire to assess your IBD activity (HBI/Partial Mayo)

Week 2, 4, 6:

- Completion of a questionnaire by phone to assess your IBD activity (HBI/Partial Mayo)

Part 2: For those completing Part 1 or those on maintenance anti-TNF therapy

Week 14:

- Complete 2 questionnaires and if a maintenance patient, also complete a demographic information sheet
- Collection of a blood sample to measure vitamin D levels & identify specific biological markers
- Collection of a urine sample
- Completion of a questionnaire to assess your IBD activity (HBI/Partial Mayo)
- **Vitamin D deficient patients will be given a vitamin D injection**

Week 18:

- Completion of a questionnaire by phone to assess your IBD activity (HBI/Partial Mayo)

Week 22:

- Complete 2 questionnaires
- Collection of a blood sample to measure vitamin D levels & identify specific biological markers
- Collection of a urine sample
- Completion of a questionnaire to assess your IBD activity (HBI/Partial Mayo)

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We will also look at your medical records to gather some information on your medical history (regarding diagnosis, treatment, extent of disease, etc.). In addition, if you are taking oral vitamin D, you must be taking 2000 IU/day or less to be included in this study.

**** Participants who complete their blood work at BioClin or another clinic in Edmonton will be required to come into the Edmonton Clinic Outpatient Laboratory for the blood sample at week 0, 14, and 22 in addition to the blood work required under your IBD standard of care.**

POSSIBLE BENEFITS
Some patients with IBD have difficulty absorbing vitamin D; therefore, vitamin D is injected as a part of IBD standard of care. It is currently not known if people with IBD taking anti-TNF therapy will receive any benefit by increasing vitamin D levels, either by taking oral tablets or by injection; however, if you receive a vitamin D injection because your levels are low, you may potentially improve your nutritional status by increasing your bone health and possibly reducing inflammation. We hope that the information we gain from this study will help doctors identify the role of vitamin D in IBD patients' response to anti-TNF induction therapy.

POSSIBLE RISKS
Venipunctures are routine procedures used to obtain blood samples, and vitamin D injections are administered under IBD standard of care to increase vitamin D levels. These are minimal risk procedures; however, possible risks include mild pain, fainting, bleeding, discoloration or bruising, and/or an infection at the place where the needle enters the skin.

CONFIDENTIALITY
Personal health records relating to this study will be kept confidential. Any research data collected about you during this study will not identify you by name, only by a coded number. Your name will not be disclosed outside the research clinic. Any report published as a result of this study will not identify you by name.

For this study, the study doctor may need to access your personal health records for health information such as past medical history and test results. He/she may also need to contact your family physician and your other health care providers to obtain additional medical information. The health information collected as part of this study will be kept confidential unless release is required by law and will be used only for the purpose of the research study. By signing the consent form, you are giving permission to the study staff to access any personally identifiable health information, which is under the custody of other health care professionals as deemed necessary for the conduct of the research.

If you chose to withdraw from the study during the study period, we will not collect new health information. You will have the option to request for withdrawal of any data or specimens during the course of the study; however, once the study period has been completed and data analysis has occurred, data can no longer be removed.

Study data will be securely stored for a minimum of 5 years at the University of Alberta, Division of Gastroenterology.

VOLUNTARY PARTICIPATION
You do not have to be in this study to receive care at this clinic. If you agree to participate in this study, you are free to withdraw from the research study at any time, and your continuing medical care will not be affected in any way. You are free to leave questions blank if you so choose. If any knowledge gained from this or any other study becomes available which could influence your decision to continue in the study, you will be promptly informed.

EXPENSE REIMBURSEMENT
No payment will be provided for your direct participation in this study.

CONTACT INFORMATION
If you have concerns about your rights as a study participant, you may contact the Research Ethics Office at 780-492-2615. This office has no affiliation with the study investigators.

Please contact the individual identified below if you have any questions or concerns
Dr. Karen Kroeker, MD, FRCPC karen.kroeker@ualberta.ca Phone 780-248-1433

APPENDIX B. Consent Form

UNIVERSITY OF ALBERTA

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CONSENT FORM

Title of Research Study:

The Role of Vitamin D in Anti-TNF Induced Remission in Patients with Inflammatory Bowel Disease

Principal Investigator: Dr. Karen Kroeker **Sub-Investigator:** Dr. Richard Fedorak
Study Coordinator: Krista Reich

	YES	NO
1) Do you understand that you have been asked to be in a research study?	☐	☐
2) Have you read and received a copy of the study information sheet?	☐	☐
3) Do you understand the benefits and risks involved in taking part in this research study?	☐	☐
4) Have you had an opportunity to ask questions and discuss this study?	☐	☐
5) Do you understand that you are free to withdraw from the study at any time without having to give a reason and without affecting your future medical care?	☐	☐
6) Has the issue of confidentiality been explained to you and do you understand who will have access to your medical records including personally identifiable information?	☐	☐

I agree to take part in this study: **YES** ☐ **NO** ☐

_____	_____	_____
Signature of Research Participant	*Date*	*Printed Name*

I believe that the person signing this form understand what is involved in the study and voluntarily agrees to participate.

_____	_____
Signature of person obtaining consent	*Date*

APPENDIX C. Demographics Form

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The Role of Vitamin D in Anti-TNF Induced Remission in Patients with Inflammatory Bowel Disease

STUDY ID

DEMOGRAPHICS SHEET
Please answer the following questions:

Gender: MALE FEMALE

Age: _____

Do you smoke currently? YES NO

Did you previously smoke? YES NO **If yes, when did you quit?** _____

How many cigarettes per day and for how many years? _____

Have you ever used recreational drugs? YES NO

Do you consume alcohol? OFTEN SOMETIMES RARELY NEVER

Are you currently pregnant? YES NO UNSURE

Do you have inflammatory bowel disease? ☐ Crohn's ☐ Ulcerative Colitis ☐ Not sure

When were you diagnosed? _____ (Year)

Have you ever had surgery for your inflammatory bowel disease? ☐ No ☐ Yes
If yes, please list the surgery and the date:

Surgery:	Date (Year):
_____	_____
_____	_____
_____	_____

Please circle any of the following medications/supplements that you do OR have taken:
IBD Medications:

Prednisone	Salofalk	Iron Pills	Vitamin B12
Budesonide/Entocort	Mezavant	Calcium	Folic Acid
Azathioprine (Imuran)	Mesasal	**Vitamin D Daily amount:** _____	
6-MP (Purinethol)	Sulfasalazine		
Methotrexate	Antibiotics: _____		
Asacol	Probiotics:VSL#3, Mutaflor (E.coli Nissle), Other:_____		
Pentasa	Omega 3/Fish Oil		

December 5, 2012 **Please complete BOTH SIDES**

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List all other medications (including non-prescription) and supplements you do OR have taken:

_____
_____
_____

CURRENT MEDICAL HISTORY:

Are you currently on any medications prescribed by a physician? YES NO
If yes, please list:

_____ _____
_____ _____
_____ _____

Do you have any other medical conditions (eg. Asthma, arthritis, heart, kidney or liver)?

YES NO

If yes, please list:

_____ _____
_____ _____
_____ _____

Have you ever had any other surgery (not related to your inflammatory bowel disease)?

YES NO

If yes, please list the surgery and the date:

Surgery:	Date (Year):
_____	_____
_____	_____
_____	_____

APPENDIX D. Harvey Bradshaw Index Questionnaire

The Role of Vitamin D in Anti-TNF Induced Remission in Patients with IBD

Modified Harvey Bradshaw Index Assessment for Crohn's Disease Activity

STUDY ID

1. General Well-being (see descriptors)
- ☐ Very well = 0
- ☐ Slightly below Par = 1
- ☐ Poor = 2
- ☐ Very Poor = 3
- ☐ Terrible = 4

☐

2. Abdominal Pain (see descriptors)
- ☐ None = 0
- ☐ Mild = 1
- ☐ Moderate = 2
- ☐ Severe = 3

☐

3. Number of Liquid or Soft Stools per day (Yesterday)

☐

Physician, please complete Question 4

4. Additional Manifestations
- ☐ None = 0
- ☐ Arthalgia = 1
- ☐ Uveitis = 1
- ☐ Erythema Nodosum = 1
- ☐ Aphtous ulcer = 1
- ☐ Pyoderma gangrenosum = 1
- ☐ Anal Fissure = 1
- ☐ New Fistula = 1
- ☐ Abscess = 1

☐

Total Harvey Bradshaw Index score: [sum of all above items]

☐

Remission = <5
Mild Disease = 5-7
Moderate Disease = 8-16
Severe Disease >16

1. General Well-being Descriptors

General well being includes fatigue in the overall rating and how you feel today. Record the worst you have felt today. Compare yourself to someone else of your age, how would they rank their general wellbeing? Below are some descriptors to help you rank your category of general well being.

- **Very Well:** General health is not generally a problem. You're feeling very good or great and under control.
- **Slightly Below Par:** You're getting through things but feeling below par and not normal. Something overall is preventing you from saying "I feel wonderful". You're feeling good but not great. You can work, socialize, and function on a day to day basis.
- **Poor:** Your symptoms bother you. You occasionally miss work, school, or social activities. You have some embarrassing moments with fecal incontinence. You have diarrhea, abdominal pain, fatigue, and basically just feeling unwell, but you are still able to function. You're getting through the day, doing all your normal stuff but it is a struggle.
- **Very Poor:** Your getting through a part of the day, but can't do you're your normal stuff. You can't attend social events in evening. You sometime leave home from work early. You feel pretty bad and are not doing much activity – only those absolutely necessary. Your symptoms interfere with life considerably, you don't go out or are fearful when out, you miss a lot of school or work. Fecal incontinence happens several times per week.
- **Terrible:** You're unable to function. You can't manage the basics and you're almost bedridden. This is the worse you have ever been. You're not working.

2. Abdominal Pain Descriptors

Abdominal pain may include cramping and discomfort. It does not have to be just "pain" as we know it. Below are some descriptors to help you rank your category of abdominal pain.

- **Mild:** You're aware that the abdominal pain is there but it does not interfere with your life and you continue with activities such as work and pleasure. You feel and hear rumbles, gurgles and cramps.
- **Moderate:** You're aware of your abdominal pain and must alter your activities to manage the pain (ie. lie down to rest, postpone shopping trips until later, and take Tylenol). The pain interferes with your life and daily activities. You may have to miss work or pleasure activities on occasion.
- **Severe:** Your abdominal pain causes you to stop all activity. You are frequently in bed because of the pain, you call in sick to work and cancel all activities.

APPENDIX E. Partial Mayo Questionnaire

The Role of Vitamin D in Anti-TNF Induced Remission in Patients with IBD

Partial Mayo Scoring Index Assessment for Ulcerative Colitis Activity

STUDY ID

Patient, please enter number of daily bowel motions you would have when in remission or before your diagnosis or symptoms of ulcerative colitis began. **This number will be Your Normal:** ☐

Patients, please complete Questions number 1 and 2.

1. **Stool Frequency (based on the past 3 days)**

Normal number of stools	= 0
1-2 stools more than normal	= 1
3-4 stools more than normal	= 2
5 or more stools more than normal	= 3

☐

2. **Rectal Bleeding (based on the past 3 days)**

No blood seen	= 0
Streaks of blood with stool less than half the time	= 1
Obvious blood with stool most of the time	= 2
Blood alone passed	= 3

☐

Physician, please complete Questions number 3.

3. **Physician's Global Assessment (to be completed by Physician)**

Normal (sub scores are mostly 0)	= 0
Mild Disease (sub scores are mostly 1)	= 1
Moderate Disease (sub scores are mostly 1 to 2)	= 2
Severe disease (sub scores are mostly 2 to 3)	= 3

☐

The physician's Global Assessment acknowledges the Sub scores, the daily record of abdominal discomfort and functional assessment and other observations such as physical findings, and the patient's performance status

Total Partial Mayo Index Score [sum of all above items] ☐

Remission = 0-1
Mild Disease = 2-4
Moderate Disease = 5-6
Severe Disease =7-9

APPENDIX F. Short Inflammatory Bowel Disease Questionnaire

Study: The Role of Vitamin D in Anti-TNF Induced Remission in Patients with IBD

Short Quality of Life in Inflammatory Bowel Disease Questionnaire

This questionnaire is designed to find out how you have been feeling during the last 2 weeks. You will be asked about symptoms you have been having as a result of your inflammatory bowel disease, the way you have been feeling in general, and how your mood has been.

1. How often has the feeling of fatigue or of being tired and worn out been a problem for you during the last 2 weeks? (Choose one)

   - ☐ All of the time
   - ☐ Most of the time
   - ☐ A good bit of the time
   - ☐ Some of the time
   - ☐ A little of the time
   - ☐ Hardly any of the time
   - ☐ None of the time

2. How often during the last 2 weeks have you had to delay or cancel a social engagement because of your bowel problem? (Choose one)

   - ☐ All of the time
   - ☐ Most of the time
   - ☐ A good bit of the time
   - ☐ Some of the time
   - ☐ A little of the time
   - ☐ Hardly any of the time
   - ☐ None of the time

3. How much difficulty have you had, as a result of your bowel problems, doing leisure or sports activities you would have liked to have done during the last 2 weeks? (Choose one)

   - ☐ A great deal of difficulty, activities made impossible
   - ☐ A lot of difficulty
   - ☐ A fair bit of difficulty
   - ☐ Some difficulty
   - ☐ A little difficulty
   - ☐ Hardly any difficulty
   - ☐ No difficulty; the bowel problems did not limit sports or leisure activities

4. How often during the last 2 weeks have you been troubled by pain in the abdomen? (Choose one)

   - ☐ All of the time
   - ☐ Most of the time
   - ☐ A good bit of the time
   - ☐ Some of the time
   - ☐ A little of the time
   - ☐ Hardly any of the time
   - ☐ None of the time

5. How often during the last 2 weeks have you felt depressed or discouraged? (Choose one)

   - ☐ All of the time
   - ☐ Most of the time
   - ☐ A good bit of the time
   - ☐ Some of the time
   - ☐ A little of the time
   - ☐ Hardly any of the time
   - ☐ None of the time

6. Overall, in the last 2 weeks, how much of a problem have you had with passing large amounts of gas? (Choose one)

   - ☐ A major problem
   - ☐ A big problem
   - ☐ A significant problem
   - ☐ Some trouble
   - ☐ A little trouble
   - ☐ Hardly any trouble
   - ☐ No trouble

7. Overall, in the last 2 weeks, how much of a problem have you had maintaining or getting to the weight you would like to be? (Choose one)

   - ☐ A major problem
   - ☐ A big problem
   - ☐ A significant problem
   - ☐ Some trouble
   - ☐ A little trouble
   - ☐ Hardly any trouble
   - ☐ No trouble

8. How often during the last 2 weeks have you felt relaxed and free of tension? (Choose one)

   - ☐ None of the time
   - ☐ A little of the time
   - ☐ Some of the time
   - ☐ A good bit of the time
   - ☐ Most of the time
   - ☐ Almost all of the time
   - ☐ All of the time

9. How much of the time during the last 2 weeks have you been troubled by a feeling of having to go to the bathroom even though your bowels were empty? (Choose one)

   - ☐ All of the time
   - ☐ Most of the time
   - ☐ A good bit of the time
   - ☐ Some of the time
   - ☐ A little of the time
   - ☐ Hardly any of the time
   - ☐ None of the time

10. How much of the time during the last 2 weeks have you felt angry as a result of your bowel problem? (Choose one)

    - ☐ All of the time
    - ☐ Most of the time
    - ☐ A good bit of the time
    - ☐ Some of the time
    - ☐ A little of the time
    - ☐ Hardly any of the time
    - ☐ None of the time

(Appendix from American Journal of Gastroenterology 1996; 91(8):1571-8.)

APPENDIX G. Beck Depression Index – Second Edition Questionnaire

BDI-II

Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness

0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all the time.
3 I am so sad or unhappy that I can't stand it.

2. Pessimism

0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. Past Failure

0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure

0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings

0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6. Punishment Feelings

0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike

0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticalness

0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes

0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. Crying

0 I don't cry anymore than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

_____ Subtotal Page 1

Continued on Back

11. Agitation

0 I am no more restless or wound up than usual.

1 I feel more restless or wound up than usual.

2 I am so restless or agitated that it's hard to stay still.

3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.

1 I am less interested in other people or things than before.

2 I have lost most of my interest in other people or things.

3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.

1 I find it more difficult to make decisions than usual.

2 I have much greater difficulty in making decisions than I used to.

3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.

1 I don't consider myself as worthwhile and useful as I used to.

2 I feel more worthless as compared to other people.

3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.

1 I have less energy than I used to have.

2 I don't have enough energy to do very much.

3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.

1a I sleep somewhat more than usual.

1b I sleep somewhat less than usual.

2a I sleep a lot more than usual.

2b I sleep a lot less than usual.

3a I sleep most of the day.

3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.

1 I am more irritable than usual.

2 I am much more irritable than usual.

3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.

1a My appetite is somewhat less than usual.

1b My appetite is somewhat greater than usual.

2a My appetite is much less than before.

2b My appetite is much greater than usual.

3a I have no appetite at all.

3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.

1 I can't concentrate as well as usual.

2 It's hard to keep my mind on anything for very long.

3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.

1 I get more tired or fatigued more easily than usual.

2 I am too tired or fatigued to do a lot of the things I used to do.

3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.

1 I am less interested in sex than I used to be.

2 I am much less interested in sex now.

3 I have lost interest in sex completely.

24 25 26 27 28 29 30 A B C D E

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