Associations between vitamin D, disease activity, and clinical response to therapy in rheumatoid arthritis

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Abstract

Objective

Vitamin D deficiency is a potential risk factor for autoimmunity. Prior studies of the association between vitamin D levels and rheumatoid arthritis (RA) disease activity have yielded conflicting results.

Methods

Serum 25(OH)vitamin D levels were measured at baseline in 499 participants with active RA, ages 18–85 years, enrolled in a randomised clinical trial of golimumab (Go-Before Trial). Subjects were methotrexate and biologic therapy naïve. Multivariable linear regression was used to assess associations between vitamin D levels and disease activity scores (DAS28), van der Heijde-Sharp (vdHS) erosion scores, and serum inflammatory markers. Generalised estimating equations were used to evaluate the associations between vitamin D status and the response to therapy over 52 weeks, using the DAS28 and ACR response.

Results

Forty-eight percent of participants were vitamin D deficient, defined as serum 25(OH)vitamin D <20 ng/mL. Deficiency was not associated with greater DAS28 (β-0.021 [95% CI -0.22, 0.18]), adjusted for age, race, sex, BMI, disease duration and glomerular filtration rate. Vitamin D deficiency was not associated with baseline vdHS scores or inflammatory markers in adjusted or unadjusted models. There was no association between baseline vitamin D deficiency and change in DAS28 (β = -0.024 [-0.30, 0.25]), proportion meeting ACR response (OR 0.82 [0.56, 1.20]), or radiographic progression at 52 weeks (OR 0.91 [0.59–1.40]).

Conclusions

Vitamin D levels were not associated with RA disease activity, inflammatory markers, or vdHS scores at baseline. Furthermore, there was no association between baseline vitamin D level and response to therapy or radiographic progression.

Key words

vitamin D, rheumatoid arthritis, disease activity, erosion, response to therapy

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Introduction

Recent data suggest that vitamin D deficiency is an important risk factor for numerous conditions including cardiovascular disease, diabetes, and certain cancers, in addition to its established role in bone and mineral metabolism (1-3). Vitamin D is a steroid hormone that also plays a key role in regulating immune cells, including macrophages and lymphocytes (4, 5). Vitamin D increases interleukin (IL)-4 production and the Th2 response *in vitro*, and may act to suppress inflammation (6). The addition of $1,25(OH)_2D$ reduced interleukin (IL)-17A and interferon-γ levels and increased IL-4 levels in stimulated peripheral blood mononuclear cells from treatment-naive patients with early rheumatoid arthritis (RA) (7).

Despite excitement in the area, the clinical literature is conflicting and may suffer from significant publication bias. Merlino *et al*. demonstrated a significantly increased incidence of RA in women from the Iowa Women's Health Study who reported less vitamin D intake (8). However, a later study by Constenbader did not confirm this (9). Small cross-sectional studies have examined the association between serum 25(OH) vitamin D levels and measures of disease activity in inflammatory arthritis, also yielding conflicting results. Patel *et al*. evaluated vitamin D levels in 206 patients with early inflammatory arthritis and found that low serum vitamin D levels were correlated with greater disease activity score 28 (DAS28), worse functional status, higher levels of inflammatory markers, and the presence of erosions at baseline (10). However, the study was limited by the heterogeneity of the population and therapies and, furthermore, the results may have been confounded by body mass, disability, and disease severity. Craig *et al*. demonstrated an inverse association between vitamin D levels and measures of disease activity among 266 African-American patients with RA but these findings did not persist when adjusted for season, age, and gender, and was not present at 3 years of follow-up (11).

A recent editorial by Welsh *et al*. summarises some of the limitations of current evidence, including use of vitamin D intake (instead of levels), reverse causality, and residual confounding (12). Multiplicity within studies and publication bias may also complicate the literature. With the recent interest in non-skeletal effects of vitamin D, more robust data are needed to clarify these associations with particular attention to hypothesis oriented approaches and controlling for potential confounding. In addition, we are aware of no previous studies that have evaluated the longitudinal response to therapy in vitamin D sufficient and deficient subjects as part of a large scale clinical trial.

This ancillary study was conducted within a random sample of 499 participants in the golimumab (Go-BEFORE) randomised controlled trial (RCT) (13). The objectives were to evaluate the association between baseline vitamin D levels and concurrent measures of disease activity, and the association between baseline levels and the response to therapy over the subsequent 52 weeks in a large cohort of subjects with an ACR diagnosis of RA, active arthritis, excellent covariable assessment, and a limited number of prior disease modifying treatments.

Methods

Study setting

Golimumab is a fully human monoclonal antibody to tumour necrosis factor alpha (TNF-α) developed for the treatment of RA. This study is ancillary to the GO-BEFORE trial (Clintrials.gov identifier NCT00361335) that included 637 RA patients with 52 weeks of follow-up. The study compared the efficacy of methotrexate or golimumab alone to combination therapy with methotrexate and golimumab. The study was carried out between December 2005 and October 2007. The trial results have already been published (13).

Patients 18 years or older were recruited from 90 sites worldwide including Asia, Europe, Australia, Latin America, and North America. Inclusion criteria included meeting ACR criteria for RA for at least the past 3 months and active disease defined by at least 4 swollen and tender joints at baseline. Participants also were required to have at least two of the following:

1. an elevated erythrocyte sedimentation rate (ESR) >28 mm/hour or C-reactive protein (CRP) >1.5 mg/dL,
2. erosions on radiographs,
3. an elevated cyclic citrullinated peptide (CCP) antibody titer or rheumatoid factor (RF),
4. the presence of morning stiffness greater than 30 minutes. Exclusion criteria included previous methotrexate or anti-TNF-α therapy.

An 80% sample was available to the investigators per company policy from the above RCT. Using a SAS-based random number generator, a random sample of 499 participants were selected from the overall group, and vitamin D levels were determined at baseline, prior to the initiation of therapy.

Vitamin D measurement

Vitamin D levels were measured in a single batch on baseline samples stored at -70°C (Heartland Assays, Ames, IA, USA). This laboratory uses a Diasorin 25(OH) vitamin D I^{125} radio-immunoassay method considered more sensitive and specific than other 25(OH) vitamin D radio-immunoassays without any significant positive or negative bias (14, 15). The antibody has equal affinity for both vitamin D_2 and vitamin D_3 and the assay has comparable measurement characteristics to liquid chromatography, tandem mass spectrometry (LC/MS) (14, 16). To define deficiency, we used a cutoff vitamin D level of <20 ng/mL (17), which is consistent with the 2010 Institute of Medicine Report (18). Serum 25(OH) vitamin D levels are the most appropriate measure of vitamin D status (19) because serum $1,25(OH)_2$ vitamin D levels are often preserved in the setting of 25(OH) vitamin D deficiency. Compensatory increase in parathyroid hormone (PTH) results in increased conversion of 25(OH) vitamin D to its activated form of $1,25(OH)_2$ vitamin D. Furthermore, recent literature suggests that 25(OH) vitamin D is substrate for intracellular synthesis of $1,25\ (OH)_2$ vitamin D in monocytes, macrophages, and other immune cells (20).

Outcome measures

Patient visits occurred at regular 4-week intervals. Data collection at each visit included independent, blinded assessments of disease activity using the DAS28 with ESR (DAS28[ESR]) and CRP (DAS28[CRP]), swollen and tender joint counts, health assessment disability questionnaires (HAQ) (21), and safety assessments. X-rays were performed at baseline, week 28, and week 52. Change from baseline in van der Heijde-Sharp (vdHS) scores at 52 weeks were determined using centralised readers and standardised methods, as previously described (13).

Our primary outcomes of interest were the DAS28(ESR) score at baseline and the change in DAS28(CRP) score over 24 and 52 weeks of follow-up (a negative value indicating improvement). DAS28(CRP) was chosen as the primary outcome for longitudinal analysis since the DAS28(ESR) was not available at all follow-up intervals.

Potential confounders and effect modifiers

Potential confounders and determinants of vitamin D status were included in the analyses. Vitamin D deficiency in the general population is associated with older age, obesity, black race, less sun exposure, physical disabilities and lower renal function (17). Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease Study Equation. Race was defined as Caucasian, Asian, Black, and other.

Five possible geographic regions were assigned to participants based on the country of measurement. These included North America, South America, Europe, Southeast Asia, and Australia. Season of measurement was determined using the hemisphere of the country and the month of measurement. November through April were considered winter months in the northern hemisphere (summer in southern hemisphere). A variable for patient-reported regular exercise was also considered as a potential correlate of vitamin D level.

For longitudinal analysis, treatment group allocation was also evaluated as a potential effect modifier.

Statistical analysis

Data were analysed on STATA 11 software. A two-tailed *p*-value <0.05 was the criterion for statistical significance. Subject characteristics were described for patients overall. Graphical displays (*e.g.* histograms, boxplots and scatter-plots) were used to examine distributions, guide data transformations as needed, and assess bivariate relations. The association between vitamin D levels and continuous variables were assessed using Spearman's rank. Mean vitamin D levels were tested for equality across categorical variables using *t*-tests (or ranksum tests for non-parametric data) and AnOVa. Variables identified as potential predictors of vitamin D levels and/or disease activity were included in the regression models. Confounding was considered to be present when the vitamin D coefficient of association changed by >15% with inclusion of the confounding variable in the model.

The primary associations of interest between vitamin D level and disease activity at baseline (as well as other outcomes) were assessed using multivariable linear regression analysis. This was performed using vitamin D as a continuous variable, as quartiles, and categorised as deficient (serum 25(OH)vitamin D level <20 ng/mL) *vs.* not deficient. Other possible confounding co-variables such as age, race, sex, BMI, season of measurement, geographic region, eGFR, presence of metabolic syndrome, patient reported exercise, and disability (HAQ) were considered in development of the model. It remains controversial whether season of measurement and geographic region should be considered confounders since they are in the causal pathway for vitamin D deficiency. Since season and geographic region were not identified as confounders of the association, they were not included in the final models presented.

Multivariable linear regression analysis was used to evaluate the association between baseline vitamin D level (per 10 ng/mL) and change in DAS28(CRP) over 24 and 52 weeks. Participants missing follow-up data were excluded from longitudinal analysis at 24 and 52 weeks. The longitudinal results include all treatment arms combined, adjusting for treatment group as a potiential confounder. Generalised Estimating Equa-

tions (GEE) were used to incorporate repeated longitudinal disease activity measures in a linear regression model. Multivariable logistic regression was performed to evaluate the association between baseline vitamin D level and binary outcomes including ACR20, ACR50, and ACR70 response and radiographic progression. Radiographic progression was defined as a change in vdHS score of >0.5.

Multivariate linear regression was used to determine predictors of low vitamin D levels, with natural log transformed 25(OH) vitamin D levels as the outcome variable. Variables were included if they were associated with vitamin D with a $p<0.2$. Stepwise deletion was performed while graphing residuals and performing Wilk tests to assess fit.

Results

Association between vitamin D levels and disease activity at baseline

Subject characteristics at enrollment are summarised in Table I in the entire sample and according to vitamin D deficiency category. Overall, 48% of participants were deficient (<20 ng/mL) at baseline. The median (inter-quartile range) vitamin D level for the entire group was 20.7 (14.5–28.2) ng/mL. Participants with vitamin D deficiency were significantly older, more likely to be female, had higher mean BMI, and had greater low-density lipoprotein (LDL) and triglycerides (TG) levels, compared with those with vitamin D levels >20 ng/mL. Disease activity, inflammatory markers, and vdHS scores were not different between groups. The vitamin D deficient participants reported marginally higher HAQ and pain scores. Geographic region, country, and season of measurement did not differ between vitamin D groups. Only 11 (2.2%) participants reported taking vitamin D supplements at baseline.

Spearman correlations did not demonstrate a significant association between vitamin D and DAS28(ESR) (Rho=-0.08 $p=0.09$), inflammatory markers, vdHS scores, or tender/swollen joint counts (all $p>0.4$).

In multivariable linear regression models, vitamin D deficiency (<20 ng/mL) was not associated with DAS28(ESR)

Table I. Baseline characteristics of patients with vitamin D levels above and below 20 ng/mL (presented as mean (SD), median (inter-quartile range), or percent. *p*-value represents the comparison between vitamin D deficiency categories.

Baseline variables	All participants	Vitamin D >20 ng/mL	Vitamin D ≤20 ng/mL	*p*-value
n. (%)	499 (100)	259 (52)	240 (48)	N/A
Age (years)	49.5 (12.4)	47.7 (11.8)	51.5 (12.6)	<0.001
Race (%)				0.03
Asian	18.4	17.0	20.0	
Caucasian	73.6	75.3	71.7	
Black	1.2	0.4	2.1	
Other	6.8	7.3	6.3	
Sex, % male	16.6	20.9	12.1	<0.01
BMI (kg/m^2)	27.2 (6.0)	26.5 (5.98)	27.9 (5.99)	<0.01
GFR (mL/min/1.75m^2)	89.3 (21.5)	88.0 (20.7)	90.8 (22.2)	0.1
Winter measurement, %	55	56	54	0.7
Diabetics, %	5.0	3.1	7.1	<0.05
Hypertension, %	27	24	31	0.07
Smoking, past or current, %	34	36	31	0.4
Regular exercise, %	13.8	15.8	11.7	0.2
Steroid use, %	53.7	54.1	53.3	0.9
Laboratory studies				
25(OH) vitamin D	21.9 (9.8)	29.3 (7.7)	14.0 (3.9)	N/A
serum albumin (g/dL)	4.2 (0.4)	4.2 (0.4)	4.2 (0.4)	0.8
low density lipoprotein	112 (33)	107 (30)	117 (35)	<0.001
triglycerides	129 (76)	119 (70)	140 (81)	<0.01
high density lipoprotein	58 (16)	58 (16)	58 (15)	0.5
Disease specific measures				
DAS28 score ESR	6.30 (1.15)	6.24 (1.24)	6.37 (1.04)	0.2
DAS28 score CRP	5.74 (1.06)	5.70 (1.09)	5.77 (1.03)	0.5
ESR (mm/hr)	38 (28, 60)	40 (25, 62)	38 (29, 60)	0.5
CRP (mg/dL)	1.3 (0.5, 3.4)	1.2 (0.5, 3.5)	1.45 (0.5, 3.35)	0.4
HAQ Score	1.56 (0.66)	1.49 (0.68)	1.62 (0.63)	<0.05
patient assessment on VAS	6.2 (4.8, 8)	5.9 (4.5, 7.8)	6.5 (4.9, 8.05)	0.05
evaluator assessment on VAS	6.2 (5.1, 7.4)	6.3 (5.1, 7.6)	6.1 (5.1, 7.3)	0.3
pain on VAS	6.65 (5.1, 8)	6.55 (4.9, 7.8)	6.7 (5.2, 8.2)	0.07
van der Heidje erosion score	4.5 (1.5, 12.5)	4.5 (1.5, 12.5)	4.5 (2, 12.5)	0.7
joint space narrowing	1 (0, 7)	1 (0, 7.8)	1 (0, 6.5)	0.8
Van der Heidje-Sharp score	6 (2, 21.5)	6 (2, 21)	6 (2, 21.75)	0.8
CCP positive %	75	77	72	0.2
disease duration (years)	1.2 (0.5, 4.2)	1.1 (0.5, 4)	1.4 (0.55, 4.55)	0.2

BMI: body mass index; GFR: glomerular filtration rate; DAS28: disease activity score 28; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; VAS: visual analogue scale; HAQ: health assessment questionnaire; CCP: cyclic citrullinated peptide.

Table II. Adjusted and unadjusted association of vitamin D category (compared to ≥30 ng/mL) with the disease activity score 28 (DAS28(ESR)) in univariable and multivariable linear regression.

Variable	β. (DAS28)	95% CI	*p*-value
Unadjusted			
Vitamin D ≥30 (n=137)	(REF)	–	–
Vitamin D 20-30 (n=103)	0.18	(-0.12, 0.48)	0.24
Vitamin D 15-20 (n=174)	0.22	(-0.12, 0.55)	0.21
Vitamin D <15 (n=85)	0.27	(-0.046, 0.59)	0.096
Adjusted*			
Vitamin D ≥30 (n=137)	(REF)	–	–
Vitamin D 20-30 (n=103)	0.11	(-0.19, 0.40)	0.47
Vitamin D 15-20 (n=174)	0.064	(-0.27, 0.39)	0.70
Vitamin D <15 (n=85)	0.032	(-0.28, 0.35)	0.84

*Adjusted for age, race, sex, BMI, disease duration, GFR, and geographic region.

(β=0.031, [-0.23, 0.17], p=0.8), adjusted for age, sex, BMI, race, disease duration, geographic region, and eGFR. Similarly, when vitamin D levels were categorised according to quartiles, there was no association with DAS28(ESR) in unadjusted or adjusted analyses (Table II). Finally, in models using continuous measures of vitamin D levels per 10 ng/mL, there was no association with DAS28(ESR) in unadjusted analyses (β=-0.098 [-0.20, 0.0056], p=0.06), or in models adjusted as above (β=0.016 [-0.12, 0.088], p=0.8). Comparable results were obtained using the DAS28(CRP) as a measures of disease activity.

Correlates of vitamin D level at baseline

Multivariate linear regression models evaluating a log-transformed vitamin D level as the outcome revealed that higher age, black race, female sex, low GFR, lack of reported regular exercise, higher serum albumin, higher LDL cholesterol, high triglycerides, and longer disease duration were significantly and independently associated with a lower vitamin D level in this cohort at baseline. When cholesterol levels were excluded from the model, higher BMI was also associated with lower vitamin D levels (p<0.02). HAQ score, pain scores, and patient reported health scores were not significantly associated with vitamin D levels at baseline.

Associations between baseline vitamin D levels and response to therapy

The 58 participants who were lost to follow-up at 52 weeks had similar baseline vitamin D levels to those with complete follow-up (21.8[9.5] *vs*. 21.9 [9.8] p=0.9). Baseline vitamin D levels did not differ across the four treatment groups in the trial.

The 52 week outcomes for patients with and without vitamin D levels <20 ng/mL at baseline are shown in Figure 1. There was no significant difference in the percent change in DAS28(CRP), CRP, swollen joint count, tender joint count, pain, or HAQ scores over the 52 weeks of follow-up. In univariable regression, there was no association between baseline vitamin D level (per 10 ng/mL) and change in DAS28(CRP) at 24 weeks (β=-0.018 [-0.15, 0.12] p=0.8) or 52 weeks (β=0.054 [-0.077, 0.19] p=0.4). The mean DAS28(CRP) score was similar at each visit in participants categorised as vitamin D deficient and sufficient based on the baseline vitamin D levels (Fig. 2). Similarly, baseline vitamin D levels were similar between EULAR responders and non-responders (p=0.3).

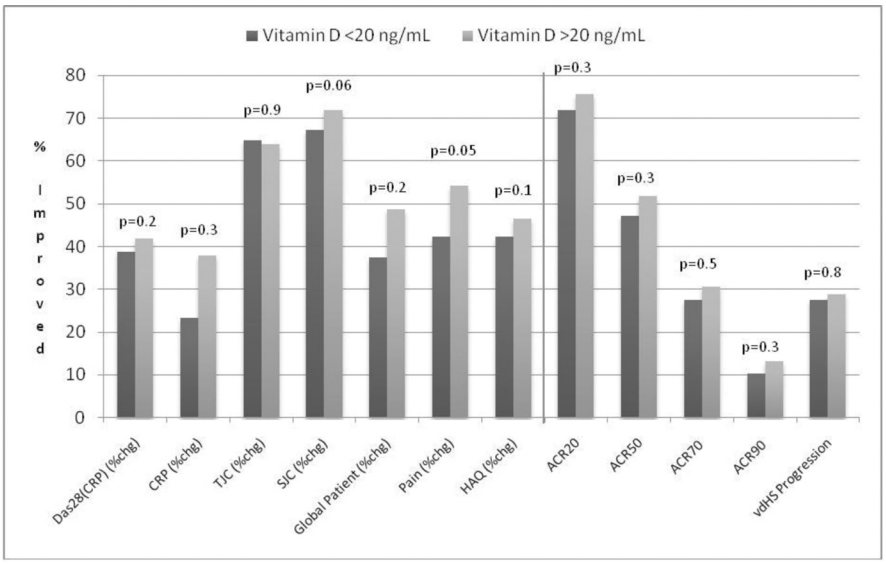

Fig. 1. Improvement in clinical outcomes at 52 weeks of follow-up by vitamin D status (greater than or less than 20 ng/mL at baseline). Continuous outcomes are displayed as percent change (%chg) and categorical variables are displayed as proportions of subjects reaching that outcome.

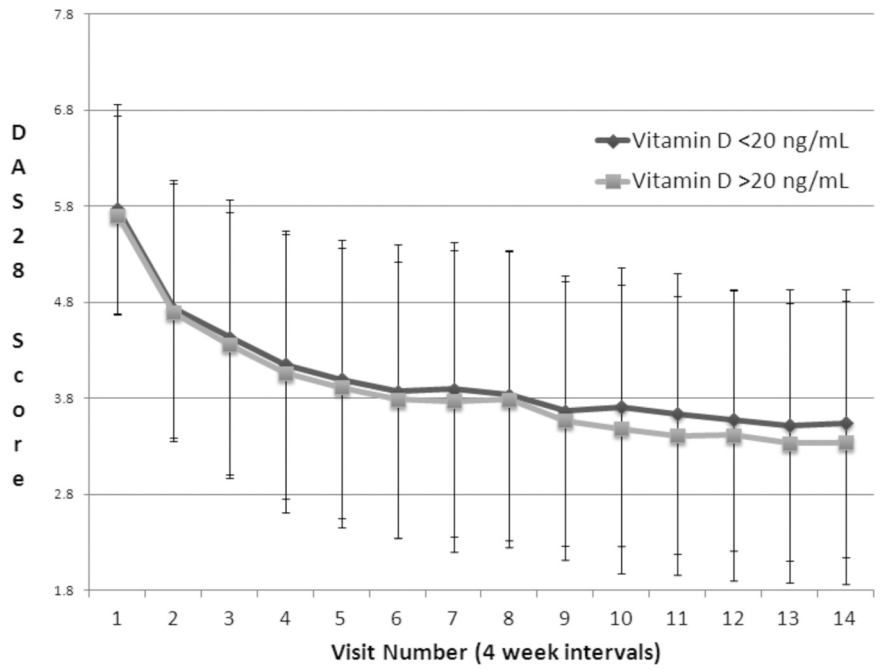

Fig. 2. Mean (SD) DAS28(CRP) score at each visit in patients with and without vitamin D deficiency defined as <20 ng/mL (n=457).

Table III summarises the multivariable linear regression models for changes in DAS28(CRP) at 24 and 52 weeks with baseline vitamin D as a continuous variable, and with baseline vitamin D categorised as <20 ng/mL *vs*. ≥20 ng/mL. In all models, baseline vitamin D levels were not correlated with change in disease activity at 24 or 52 weeks of follow-up. At 24 and 52 weeks, where DAS28(ESR) was also available, the

Table III. Multivariable linear regression analysis of the association between vitamin D level and the change in DAS28(CRP) score from the initiation of therapy at 24 weeks, 52 weeks, and in GEE analysis incorporating all DAS28(CRP) scores (12) at 4-week intervals.

Interval	β. (DAS28)*	95% CI	*p*-value
24 weeks (n=471)			
per 10 ng/ml	-0.025	(-0.16, 0.11)	0.7
<20 ng/mL	0.057	(-0.21, 0.32)	0.7
52 weeks (n=441)			
per 10 ng/mL	0.063	(-0.073, 0.20)	0.4
<20 ng/mL	-0.012	(-0.26, 0.28)	0.9
GEE (n=497)			
per 10 ng/mL	-0.019	(-0.11, 0.077)	0.7
<20 ng/mL	0.022	(-0.17, 0.21)	0.8

*Adjusted for age, sex, race, BMI, disease activity at baseline, disease duration, randomisation group, GFR, and geographic region. GEE: generalised estimating equations.

results were similar. GEE analysis, which enabled the incorporation of all DAS28(CRP) measures at 4-week intervals over the 52 weeks of follow-up into a single analysis, also did not show any association between baseline vitamin D level and the change in DAS28(CRP) score. Similarly, there were no differences in DAS28 response by quartile of vitamin D level (not shown).

In multivariable logistic regression, there were no independent associations between baseline vitamin D level (per 10 ng/ml) and ACR20, ACR50, ACR70, or ACR90 response at 52 weeks. Vitamin D deficiency (<20 ng/mL) was not associated with a significantly lower likelihood of meeting ACR50 response, the primary outcome of the original trial (OR 0.82 [0.56, 1.20] p=0.3). Additionally, there was no association between baseline vitamin D (per 10 ng/mL) and the change in inflammatory markers (ESR/CRP) or the change in self-reported measures such as pain, patient global health, or the HAQ score. Vitamin D deficiency (<20 ng/mL) was not associated with an increased risk of radiographic progression in a logistic regression model after adjustment for age, sex, race, DAS28(ESR) at baseline, treatment group, disease duration at baseline, season, and baseline vdHS score (OR 0.91 [0.59, 1.41] p=0.7). Similarly, the probability of response was not different between quartiles of vitamin D levels (not shown).

There was no evidence of modification of the effect of vitamin D by treatment group in any of the above regression models.

Discussion

This study, conducted in 499 participants with active RA enrolled in the golimumab (Go-Before) RCT, demonstrated that vitamin D levels are not associated with disease activity at enrollment as measured by DAS28 in unadjusted or adjusted analyses. Importantly, the upper bound of the 95% confidence interval for the difference in DAS28 between vitamin D deficient and vitamin D sufficient participants was 0.18, indicating that these data were not consistent with a greater than 0.18 higher disease activity score in vitamin D deficient patients. Similarly, vitamin D levels were not associated with inflammatory markers, swollen and tender joints, or erosion scores. Therefore, this well-powered study conducted in a well-characterised sample of patients with active RA does not support the hypothesis that vitamin D deficiency is related to disease activity prior to initiation of treatment.

Prior cross-sectional studies of the association between vitamin D levels and disease activity among patients with RA produced conflicting results. However, these studies may have been confounded by risk factors for vitamin D deficiency. Our data demonstrated that vitamin D deficiency was associated with older age, black race, female sex, low GFR, lack of reported regular exercise, and greater RA disease duration. A prior study in 266 African-American patients with RA demonstrated that the inverse association between vitamin D levels and measures of disease activity did not persist when adjusted for season, age, and sex (11).

To our knowledge, this is the first study to examine the association between baseline vitamin D status and subsequent changes in disease activity. These data demonstrate that baseline vitamin D levels were not associated with changes in any measures of disease activity, including DAS28(ESR), DAS28(CRP), ACR response, or radiographic progression at 24 or 52 weeks of effective RA therapy. Again, the upper bound of the 95% confidence interval suggests that vitamin D deficiency was associated with no more than 0.28 lesser decline in DAS28 at 52 weeks (Table III); this is not a clinically significant difference in disease activity over this interval. These observation data suggest that vitamin D deficiency is not associated with refractory disease or a poorer response to medical therapy.

There are several limitations to our study. First, the greatest limitation is the observational design. A RCT is necessary to definitively determine if vitamin D supplementation and greater levels are associated with lesser disease activity and a greater response to therapy. Second, there were few patients of African descent, the population at highest risk for deficiency, and thus our results may not be generalisable to this group. However, this study population was noted to have a high prevalence of deficiency, despite having few black patients. Third, this cohort was selected to have high disease activity as part of a clinical trial; potentially resulting in a more narrow range of disease activity than other studies. However, we did have a relatively wide and normally distributed range of DAS28 scores. Other groups have found associations in cohorts where a greater proportion of subjects were in remission (22). Finally, the longitudinal data were based on a single baseline 25(OH) vitamin D level. Repeated measures of vitamin D and measurement of parathyroid hormone may have provided additional information (23).

In conclusion, our observational study strongly suggests that vitamin D levels are not associated with disease activity or other measures of inflammatory activity in patients with RA, and are not associated with the response to therapy

or radiographic progression. Future intervention studies are needed to determine if vitamin D deficiency contributes to immune function and disease activity in RA.

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