ORIGINAL ARTICLE

High-Dose Vitamin D_3 during Tuberculosis Treatment in Mongolia
A Randomized Controlled Trial

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Abstract

Rationale: Existing trials of adjunctive vitamin D in the treatment of pulmonary tuberculosis (PTB) are variously limited by small sample sizes, inadequate dosing regimens, and high baseline vitamin D status among participants. Comprehensive analyses of the effects of genetic variation in the vitamin D pathway on response to vitamin D supplementation are lacking.

Objectives: To determine the effect of high-dose vitamin D_3 on response to antimicrobial therapy for PTB and to evaluate the influence of single-nucleotide polymorphisms (SNPs) in vitamin D pathway genes on response to adjunctive vitamin D_3.

Methods: We conducted a clinical trial in 390 adults with PTB in Ulaanbaatar, Mongolia, who were randomized to receive four biweekly doses of 3.5 mg (140,000 IU) vitamin D_3 (n = 190) or placebo (n = 200) during intensive-phase antituberculosis treatment.

Measurements and Main Results: The intervention elevated 8-week serum 25-hydroxyvitamin D concentrations (154.5 nmol/L vs. 15.2 nmol/L in active vs. placebo arms, respectively; 95% confidence interval for difference, 125.9–154.7 nmol/L; $P < 0.001$) but did not influence time to sputum culture conversion overall (adjusted hazard ratio, 1.09; 95% confidence interval, 0.86–1.36; $P = 0.48$). Adjunctive vitamin D_3 accelerated sputum culture conversion in patients with one or more minor alleles for SNPs in genes encoding the vitamin D receptor (rs4334089, rs11568820) and 25-hydroxyvitamin D 1α-hydroxylase (*CYP27B1*: rs4646536) (adjusted hazard ratio ≥ 1.47; P for interaction ≤ 0.02).

Conclusions: Vitamin D_3 did not influence time to sputum culture conversion in the study population overall. Effects of the intervention were modified by SNPs in *VDR* and *CYP27B1*.

Clinical trial registered with www.clinicaltrials.gov (NCT01657656).

Keywords: host-directed therapy; single-nucleotide polymorphisms; pharmacogenetics

Vitamin D was used to treat pulmonary tuberculosis (PTB) in the preantibiotic era (1). Its major circulating metabolite, 25-hydroxyvitamin D (25[OH]D), supports innate antimicrobial immune responses, suggesting a potential mechanism by which adjunctive vitamin D might enhance response to antituberculous therapy (2). With one exception (3), randomized controlled trials (RCTs) evaluating effects of vitamin D supplementation on sputum culture

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At a Glance Commentary

Scientific Knowledge on the Subject: Existing trials of adjunctive vitamin D in pulmonary tuberculosis treatment are variously limited by small sample sizes, inadequate dosing regimens, and high baseline vitamin D status among participants. Comprehensive analyses of the effects of genetic variation in the vitamin D pathway on response to vitamin D supplementation are lacking.

What This Study Adds to the Field: This is the largest clinical trial to investigate effects of adjunctive vitamin D on sputum culture conversion in patients with pulmonary tuberculosis and the first such study to systematically investigate the influence of genetic variation in the vitamin D pathway on patients' response to vitamin D supplementation. It used a high dose of vitamin D in a population with high rates of profound vitamin D deficiency at baseline. Adjunctive vitamin D did not accelerate sputum culture conversion in patients with drug-sensitive pulmonary tuberculosis, despite favorable modulation of host immune responses. We confirmed our previous finding that genetic variation in the gene encoding the vitamin D receptor modifies the effects of adjunctive vitamin D and extend this to show for the first time that a polymorphism in the gene encoding the 25-hydroxyvitamin D 1α-hydroxylase enzyme CYP27B1 is similarly effect modifying.

conversion during intensive-phase therapy have not demonstrated effects on this outcome (4–7). However, these trials are variously limited by low prevalence of vitamin D deficiency in the study population at baseline, modest or unmeasured 25(OH)D response to supplementation, significant loss to follow-up, and low statistical power. Moreover, no trial conducted to date has investigated the influence of mutations in genes other than that encoding the vitamin D receptor (VDR) on responses to vitamin D supplementation in PTB, despite evidence that single nucleotide polymorphisms (SNPs) in other genes in the vitamin D pathway influence disease outcomes (8). We therefore conducted an RCT that was powered to detect a modest effect of high-dose vitamin D on time to sputum culture conversion in a population where profound deficiency was highly prevalent, with prespecified subgroup analysis to evaluate the effect of 36 SNPs in 11 vitamin D pathway genes on the response to vitamin D supplementation. Some of the results of this study have been previously reported in the form of abstracts (9, 10).

Methods

Study Design

We conducted a two-arm parallel double-blind randomized placebo-controlled trial in the tuberculosis clinic at the National Center for Communicable Disease in Ulaanbaatar, Mongolia. The study was approved by the Ethics Review Board of the Mongolian Ministry of Health and the Office of Human Research Administration, Harvard School of Public Health (reference 19435). The trial was registered with ClinicalTrials.gov (NCT01657656).

Participants

Adults with newly diagnosed PTB and acid-fast bacilli (AFB) visible on sputum smear microscopy were assessed for eligibility to participate. Principal exclusion criteria were age younger than 18 years and serum corrected calcium concentration greater than 2.65 mmol/L; full details of eligibility criteria are presented in the online supplement. Written informed consent was obtained from all participants before enrolment.

Randomization and Masking

Patients were randomly assigned to receive either four oral doses of 3.5 mg (140,000 IU) vitamin D_3 (each dose given as four tablets each containing 875 μg [35,000 IU] vitamin D_3) or placebo (each dose given as four placebo tablets of identical appearance and taste to active study medication), with a one-to-one allocation ratio. Active and placebo tablets were manufactured by Tishcon Corp., Westbury, NY. Full details of randomization methods are presented in the online supplement. Treatment allocation was concealed from patients and study staff. Those analyzing the data were not masked to group assignment.

Procedures

All participants initially received intensive-phase antimicrobial therapy comprising isoniazid, rifampicin, pyrazinamide, and ethambutol. Those who were subsequently found to have a multidrug-resistant (MDR) isolate were switched from first-line therapy to an appropriate MDR TB treatment regimen according to the World Health Organization MDR TB guideline that was extant at the time of the study (11). The first dose of study medication was administered within 2 days of starting antimicrobial treatment, and subsequent doses were administered at 2, 4, and 6 weeks after the start of antimicrobial treatment. Study medication was discontinued if participants withdrew consent to participate in the study, if they discontinued anti-TB treatment, if they were unable to provide a sputum sample, or if they developed drug-induced liver injury or hypercreatininemia. After completion of 8 weeks of antimicrobial therapy, participants were discharged from the study, and continuation-phase antimicrobial therapy was initiated.

Participants completed a baseline clinical assessment including chest radiography; measurement of height, weight, and mid-upper arm circumference; and triceps, biceps, and subscapular skinfold thicknesses. Chest radiographs were read independently by two radiologists who were blinded to participant allocation; presence versus absence of cavities was recorded, along with the number of lung zones affected (nine zones per lung). Body mass index (BMI) was calculated using the formula: BMI = weight (kg)/[height (m)]^2. We also collected a sputum sample for microscopy and culture, a urine sample for determination of urinary calcium:creatinine ratio, and a blood sample for DNA extraction and genotyping and determination of full blood count, erythrocyte sedimentation rate (ESR), and serum concentrations of calcium, albumin, C-reactive protein (CRP), and 25(OH)D. Participants were reviewed at 2, 4, 6, and 8 weeks after starting antituberculous therapy to assess clinical status and to monitor for adverse events. At each time point, a sputum specimen was collected from all patients who were able to expectorate spontaneously, weight and skin-fold

thicknesses were measured, and urine and blood samples were collected as above. Sputum samples were transported at ambient temperature to the Reference Laboratory at the Tuberculosis Surveillance Centre of the Mongolian National Center for Communicable Disease. Chest radiography was repeated at 8 weeks. Full details of all laboratory methods are presented in the online supplement.

Outcomes

The primary outcome of the study was time from initiation of antituberculous therapy to sputum culture conversion, estimated as the midpoint between the last positive sputum culture and the first negative sputum culture thereafter. Participants who were unable to expectorate spontaneously were deemed to be culture negative. Where participants were culture positive at their last follow-up (either at 8 weeks or earlier if lost to follow-up), time to sputum culture conversion was attributed as the number of days from the date of treatment initiation to the date of the last follow-up visit, and the censor variable was assigned a zero value to indicate that the endpoint of sputum culture conversion was not achieved. Secondary outcomes were the proportion of participants with negative sputum culture at 8 weeks; time from initiation of antituberculous therapy to sputum smear conversion (estimated as the midpoint between the last positive sputum smear and the first negative sputum smear thereafter); mean number of zones affected on chest radiograph at 8 weeks; mean serum concentrations of 25(OH)D, corrected calcium, CRP, and albumin; mean ESR, total white blood cell count, neutrophil count, monocyte count, and lymphocyte:monocyte ratio in peripheral blood; and mean BMI, mid-upper arm circumference, triceps skin-fold thickness, and scapular skin-fold thickness. Safety was assessed by monitoring incidence of all serious adverse events (including deaths) and nonserious adverse events potentially related to the intervention (hypercalcemia and hypercalciuria).

Sample Size and Statistical Analysis

Assuming a median time to sputum culture conversion of 7 weeks in the control group, a follow-up period of 8 weeks, and accrual time of 2 years, we calculated that a total of 278 patients (139 patients in each group) would need to be recruited to detect a 2-week difference in median time to culture conversion between intervention and control groups with 80% power using a two-sided test at the 5% significance level (12). This number was increased by 25% to compensate for potential loss to follow-up, giving an original target sample size of 348. An additional 42 participants were enrolled for investigation of additional immunological outcomes, bringing total sample size to 390. Efficacy analysis was by intention to treat. All participants who took at least one dose of study medication were included in the safety analysis. One prespecified interim analysis at 12 months was performed by members of the Data Safety and Monitoring Board to test the effect of allocation on the primary outcome and incidence of serious adverse events. The Board recommended that the trial should continue, and results were not shared with investigators.

Analyses were performed using the STATA/IC (version 12.1, 2012; College Station, TX) software package. Significance was tested at the 5% level. The primary analysis for effect of allocation on time to sputum culture conversion was conducted using a Cox proportional hazards regression model adjusted for presence versus absence of cavitation on baseline chest radiograph (the stratification factor). A prespecified secondary analysis also adjusted for factors associating with delayed sputum culture conversion on univariate analysis with $P < 0.20$ (*see* Table E1 in the online supplement): age, sex, baseline sputum smear (<100 vs. ⩾100 AFB per 100 high-power fields), drug-sensitivity profile (MDR TB vs. not), current smoking (yes vs. no), and current alcohol use (yes vs. no). Time to sputum smear conversion was similarly analyzed. The proportion of participants with sputum culture conversion at 8 weeks was analyzed with logistic regression adjusted for the factors listed above. Continuous outcome measures assessed at baseline and 2-weekly intervals thereafter were analyzed using linear regression adjusted for the factors listed above with random effects of individual, constrained so that there was no treatment effect at baseline, and with a treatment effect estimated at each subsequent time point. A P value for allocation–time interaction was used to evaluate evidence for an effect of allocation; where evidence was found ($P < 0.05$), adjusted effect estimates with 95% confidence intervals (CIs) at individual time points are reported. Subgroup analyses were performed by repeating primary efficacy analyses with the inclusion of the appropriate interaction term. Interaction

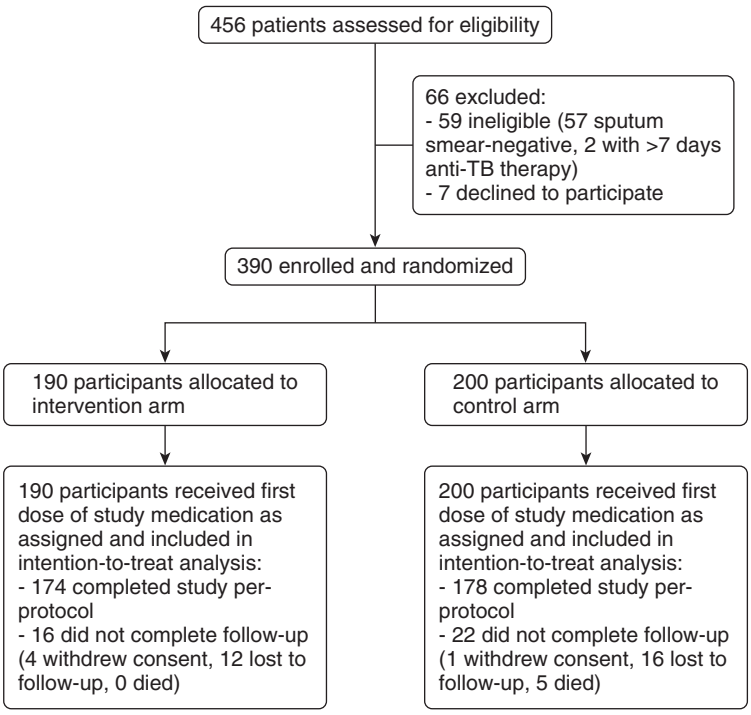

Figure 1. Trial profile. TB = tuberculosis.

effects were summarized as a ratio of hazard ratios (HRs) with 95% CI and *P* value. The Benjamini-Hochberg procedure for multiple-testing correction was applied to genetic analyses to control the false discovery rate at 20% (13).

Results

Participant Flow and Recruitment

Participant flow is illustrated in Figure 1. A total of 456 patients were assessed for eligibility to participate in the trial between October 31, 2012 and March 21, 2014: 66 were ineligible and 390 were randomized, of whom 190 were allocated to vitamin D and 200 were allocated to placebo. The trial ended on the date of the final study visit of the last participant to be randomized. All randomized participants received at least one dose of study medication and were included in the intention-to-treat analysis.

Baseline Characteristics

Participants' clinical and demographic characteristics were comparable for intervention versus control groups at baseline (Table 1). The majority of participants were profoundly vitamin D deficient (serum 25[OH]D concentration < 25 nmol/L in 311 of 384 [81%] for whom baseline measurements were available), and almost all (373 of 384 [97%]) had serum 25[OH]D concentrations less than 75 nmol/L at baseline.

Table 1. Baseline Characteristics by Allocation

	Vitamin D (*n* = 190)	Placebo (*n* = 200)
Median age, yr, IQR	31 (23–44)	35 (25–47)
Female	67 (35.3)	67 (33.5)
Ethnic group		
Khalkh	174 (91.6)	179 (89.5)
Other	16 (8.4)	21 (10.5)
Housing		
Own house/apartment	156 (82.1)	172 (86.0)
Renting room, staying with relatives, other	34 (17.9)	28 (14.0)
Currently smoking	74 (39.0)	99 (49.5)
Drinks any alcohol	81 (42.6)	102 (51.0)
Diabetes mellitus	9 (4.7)	10 (5.0)
Sputum smear		
<100 AFB per 100 high-power fields	52 (27.4)	60 (30.0)
⩾100 AFB per 100 high-power fields	138 (72.6)	140 (70.0)
Drug susceptibility of isolate		
Fully sensitive	146 (76.8)	157 (78.5)
Multidrug resistant	13 (6.8)	8 (4.0)
Isoniazid monoresistant	30 (15.8)	34 (17.0)
Rifampicin monoresistant	1 (0.5)	1 (0.5)
Serum 25(OH)D concentration, nmol/L*		
All participants, mean (SD)	7.8 (11.8)	6.0 (7.2)
<10, n (%)	66 (35.7)	61 (30.7)
10.0–24.9, n (%)	75 (40.5)	109 (54.8)
25.0–49.9, n (%)	30 (16.2)	21 (10.6)
50–74.9, n (%)	5 (2.7)	6 (3.0)
⩾75, n (%)	9 (4.9)	2 (1.0)
Serum corrected calcium, mmol/L	2.28 (0.16)	2.26 (0.18)
Urinary calcium:creatinine molar ratio	0.32 (0.25)	0.39 (0.39)
Hemoglobin, g/dl	12.9 (1.9)	13.0 (2.0)
Total white blood cell count $\times 10^9$/L	8.9 (3.1)	8.9 (3.3)
Neutrophil count $\times 10^9$/L	6.3 (2.8)	6.3 (3.0)
Lymphocyte count $\times 10^9$/L	1.5 (0.7)	1.5 (0.6)
Monocyte count $\times 10^9$/L	0.89 (0.35)	0.89 (0.39)
Erythrocyte sedimentation rate, mm/h	17.2 (10.7)	15.8 (11.3)
C-reactive protein, mg/L	62.7 (46.1)	63.0 (46.7)
Body mass index, kg/m^2	19.7 (2.8)	20.1 (3.1)
Baseline chest radiograph		
Cavities present	95 (50.0)	100 (50.0)
Zones affected (range, 1–18)	7.4 (4.4)	7.3 (4.4)

Definition of abbreviations: 25(OH)D = 25-hydroxyvitamin D; AFB = acid-fast bacilli; IQR = interquartile range.
Data are n (%) or mean (SD) except where stated otherwise.
*Results missing for five participants in the vitamin D arm and one participant in the placebo arm.

Primary Outcome

The median time to sputum culture conversion was 35 days (interquartile range, 21.5–49.5 d) in the intervention arm and 35.5 days (95% CI, 31.0–50.0 d) in the control arm (Figure 2A; Table 2). The HR for effect of allocation on time to sputum culture conversion, adjusted for presence versus absence of cavitation on baseline chest radiograph in the Cox regression model, was 1.09 (95% CI, 0.86–1.36; $P = 0.48$).

Male sex, age greater than or equal to the median value of 34 years, presence of cavitation on baseline chest radiograph, presence of greater than 100 AFB per 100 high-power fields on baseline sputum smear, presence of MDR isolate, current cigarette smoking (any vs. none), and current alcohol intake (any vs. none) were associated with delayed sputum culture conversion on univariate analysis, with $P < 0.20$ (Table E1). The HR for effect of allocation on time to sputum culture conversion after adjustment for all of these factors in the Cox regression model was 1.11 (95% CI, 0.88–1.39; $P = 0.39$; Table 2).

Secondary Outcomes, Efficacy

Results from analysis of secondary efficacy outcomes are presented in Table 2. Allocation to vitamin D versus placebo did not influence the proportion of participants with sputum culture conversion at 8 weeks (150 of 190 vs. 148 of 200, respectively; adjusted odds ratio, 1.47; 95% CI, 0.88–2.45; $P = 0.14$). Vitamin D did, however, accelerate sputum smear conversion (adjusted HR, 1.47; 95% CI, 1.09–1.98; $P = 0.01$; Figure 2B) and radiographic resolution (mean number of zones affected on chest radiograph at 8 weeks, 5.48 in intervention arm vs. 5.69 in placebo arm; 95% CI for difference, 0.06–0.77 zones; $P = 0.02$). The regimen of vitamin D administered in the trial was highly effective in correcting vitamin D deficiency in the intervention arm: mean serum 25(OH)D concentration at 8 weeks was 154.5 nmol/L in the intervention arm of the study versus 15.2 nmol/L in the control arm (95% CI for difference, 125.9–154.7 nmol/L; $P < 0.001$). Allocation to the intervention arm of the study also induced an increase in mean serum

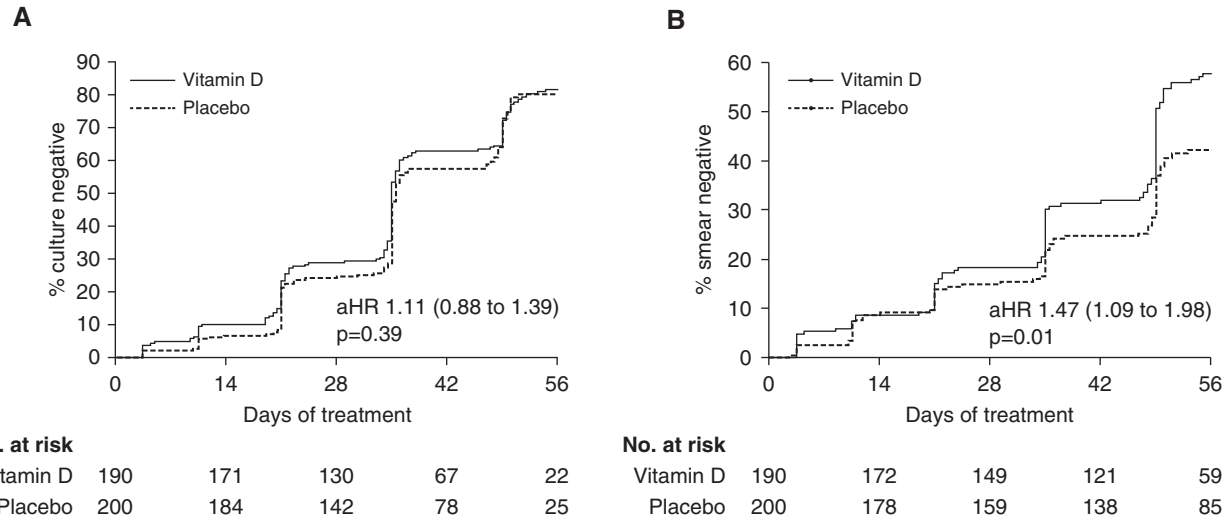

Figure 2. Time to sputum culture conversion (*A*) and smear conversion (*B*) by allocation. The number of participants with positive sputum culture/smear remaining in follow-up (number at risk) after 0, 14, 28, 42, and 56 days of antimicrobial therapy is presented. aHR = adjusted hazard ratio.

corrected calcium concentration and mean urinary calcium:creatinine ratio from the 2-week time point onward ($P < 0.001$). No consistent effect of vitamin D supplementation was seen on serum concentrations of the acute-phase reactants CRP, albumin, or ESR. However, administration of vitamin D reduced total white blood cell counts, neutrophil counts, and monocyte counts and elevated lymphocyte:monocyte ratios in peripheral blood. No clinically significant effect of vitamin D was seen on anthropometric outcomes.

Subgroup Analyses

Results of subgroup analyses are presented in Table 3. The effect of vitamin D supplementation was not modified by participants' baseline vitamin D status, sex, age, presence versus absence of cavitation on baseline chest radiograph, degree of smear positivity at baseline, or adherence to the trial protocol. Of the 21 participants with MDR TB at baseline, 5 of 13 randomized to vitamin D were sputum culture negative at 8 weeks versus 0 of 8 randomized to placebo ($P = 0.11$; Fisher exact test). Genetic subgroup analyses revealed two SNPs in VDR (rs4334089 and rs11568820) and one SNP in cytochrome p450 27B1 (*CYP27B1*) (rs4646536) that significantly modified the effect of vitamin D supplementation on time to sputum culture conversion after correction for multiple comparisons (P for interaction ≤ 0.02). In all three cases, vitamin D supplementation accelerated sputum culture conversion in individuals carrying the minor allele of each polymorphism (adjusted HR ≥ 1.47), but not in those who were homozygous for the major allele.

Adverse Events

Details of serious adverse events (SAEs) arising during the trial are presented in Table E2. Seven SAEs were observed in 7 out of the 200 participants who received at least one dose of placebo; one SAE was observed in 1 out of the 190 participants who received at least one dose of vitamin D. Five patients died during the study, all in the placebo arm. No SAE was judged to be potentially related to study medication. Details of nonserious adverse events arising during the trial are presented in Table E3. Hypercalcemia was recorded at least once in 10 of 190 participants in the intervention arm versus 5 of 200 participants in the control arm. This was asymptomatic in all cases, and it resolved spontaneously without specific treatment. Hypercalciuria was recorded at least once in 75 of 190 participants in the intervention arm versus 30 of 200 participants in the control arm; hypocalcaemia was recorded at least once in 96 of 190 participants in the intervention arm versus 160 of 200 in the control arm.

Discussion

To our knowledge, this study is the largest RCT to investigate effects of adjunctive vitamin D supplementation on the outcome of sputum culture conversion in patients with PTB to be performed to date. It is also the only such study to comprehensively investigate the influence of genetic variation in the vitamin D pathway on PTB patients' response to adjunctive vitamin D. In the study population as a whole, adjunctive vitamin D did not influence time to sputum culture conversion (the primary outcome), despite accelerating sputum smear conversion and exerting favorable immunomodulatory actions. No deaths occurred in the intervention arm, but five deaths occurred in the placebo arm. Our genetic analysis identified novel variants in VDR and *CYP27B1* that modified the effect of the intervention: adjunctive vitamin D accelerated sputum culture conversion in patients carrying one or more minor alleles of these SNPs but not in those homozygous for the major alleles.

Our null finding with respect to the outcome of sputum culture conversion is consistent with those of other trials in the field conducted by ourselves (4) and others (5–7), with one exception (3). We also replicated previous findings that adjunctive vitamin D exerted antiinflammatory actions (accelerating resolution of peripheral blood neutrophilia) and boosted the lymphocyte: monocyte ratio in peripheral blood (14). The latter marker has been identified as a biomarker of resolution of pulmonary inflammation in an animal model of TB (15). Taking these findings together

Table 2. Outcome Measures by Allocation

	Time Point (*wk*)	Vitamin D (*n = 190*)	Placebo (*n = 200*)	Adjusted Hazard Ratio (95% CI)*	Adjusted Odds Ratio (95% CI)*	Adjusted Mean Difference (95% CI)*	*P* Value
Time to sputum culture conversion, d, median (IQR)	—	35.0 (21.5 to 49.5)	35.5 (31.0 to 50.0)	1.11 (0.88 to 1.39)	—	—	0.39
Proportion with sputum culture conversion, n (%)†	8	150/183 (82.0)	148/183 (80.9)	—	1.28 (0.72 to 2.30)	—	0.40
Proportion with sputum culture conversion, n (%)‡	8	150/190 (79.0)	148/200 (74.0)	—	1.47 (0.88 to 2.45)	—	0.14
Time to sputum smear conversion, d, median (IQR)§	—	49.0 (35.0 to >56)	>56 (47.0 to >56)	1.47 (1.09 to 1.98)	—	—	0.01
Chest radiograph: No. of zones affected	8	5.48 (4.36)	5.69 (4.48)	—	—	−0.42 (−0.77 to −0.06)	0.02
Serum 25(OH)D concentration, nmol/L	2	79.3 (19.0)	7.0 (11.9)	—	—	73.3 (58.9 to 87.7)	<0.001‖
	4	115.3 (21.5)	10.3 (30.2)	—	—	106.0 (91.6 to 120.3)	
	6	146.1 (24.1)	17.2 (24.4)	—	—	129.9 (115.5 to 144.3)	
	8	154.5 (38.8)	15.2 (13.1)	—	—	140.3 (125.9 to 154.7)	
Serum corrected calcium concentration, mmol/L	2	2.34 (0.20)	2.25 (0.18)	—	—	0.09 (0.05 to 0.12)	<0.001‖
	4	2.34 (0.17)	2.25 (0.18)	—	—	0.09 (0.05 to 0.12)	
	6	2.31 (0.16)	2.23 (0.16)	—	—	0.08 (0.04 to 0.12)	
	8	2.30 (0.15)	2.22 (0.17)	—	—	0.08 (0.05 to 0.12)	
Urinary calcium: creatinine molar ratio	2	0.64 (0.44)	0.41 (0.32)	—	—	0.23 (0.08 to 0.38)	0.002‖
	4	0.71 (0.53)	0.40 (0.33)	—	—	0.32 (0.17 to 0.47)	
	6	0.72 (0.55)	0.42 (0.38)	—	—	0.30 (0.14 to 0.45)	
	8	0.71 (0.55)	0.55 (2.03)	—	—	0.16 (0.01 to 0.32)	
Serum C-reactive protein concentration, mg/L	2	43.0 (41.7)	33.5 (33.8)	—	—	9.8 (4.0 to 15.6)	0.01‖
	4	34.0 (35.0)	32.6 (31.6)	—	—	2.1 (−3.9 to 8.0)	
	6	24.0 (23.5)	31.2 (32.3)	—	—	−6.7 (−12.7 to −0.7)	
	8	20.2 (24.6)	25.2 (28.2)	—	—	−4.4 (−10.5 to 1.6)	
Serum albumin concentration, g/L	2	36.2 (5.7)	36.6 (6.0)	—	—	—	0.06‖
	4	38.5 (6.6)	38.1 (6.3)	—	—	—	
	6	40.2 (6.1)	39.2 (6.1)	—	—	—	
	8	41.0 (5.8)	40.4 (5.3)	—	—	—	
Total white blood cell count $\times 10^9$/L	2	8.8 (3.0)	8.4 (3.4)	—	—	0.4 (−0.1 to 0.9)	0.005‖
	4	8.8 (3.2)	8.7 (4.2)	—	—	0.1 (−0.4 to 0.7)	
	6	8.1 (2.8)	8.3 (3.2)	—	—	−0.3 (−0.8 to 0.2)	
	8	7.4 (2.3)	8.1 (3.0)	—	—	−0.8 (−1.3 to −0.2)	
Neutrophil count $\times 10^9$/L	2	6.1 (2.9)	5.8 (3.2)	—	—	0.3 (−0.1 to 0.8)	0.001‖
	4	6.0 (3.1)	5.9 (3.3)	—	—	0.1 (−0.3 to 0.6)	
	6	5.3 (2.7)	5.6 (3.0)	—	—	−0.3 (−0.8 to 0.1)	
	8	4.6 (2.0)	5.4 (2.7)	—	—	−0.8 (−1.3 to −0.3)	
Monocyte count $\times 10^9$/L	2	0.83 (0.35)	0.78 (0.29)	—	—	0.06 (0.01 to 0.11)	0.012‖
	4	0.81 (0.33)	0.83 (0.36)	—	—	−0.01 (−0.06 to 0.05)	
	6	0.75 (0.29)	0.79 (0.33)	—	—	−0.04 (−0.09 to 0.02)	
	8	0.71 (0.30)	0.78 (0.31)	—	—	−0.06 (−0.12 to −0.01)	
Lymphocyte count $\times 10^9$/L	2	1.6 (0.6)	1.6 (0.6)	—	—	—	0.12‖
	4	1.8 (0.7)	1.6 (0.6)	—	—	—	
	6	1.8 (0.7)	1.7 (0.6)	—	—	—	
	8	1.8 (0.6)	1.8 (0.7)	—	—	—	
Lymphocyte:monocyte ratio	2	2.2 (1.2)	2.2 (1.1)	—	—	0.0 (−0.2 to 0.2)	0.001‖
	4	2.6 (1.5)	2.2 (1.1)	—	—	0.3 (0.1 to 0.5)	
	6	2.7 (1.4)	2.5 (1.4)	—	—	0.2 (0.0 to 0.4)	
	8	2.9 (1.2)	2.5 (1.3)	—	—	0.4 (0.2 to 0.6)	
Erythrocyte sedimentation rate, mm/h	2	15.0 (11.5)	14.7 (11.8)	—	—	—	0.84‖
	4	13.9 (10.7)	12.7 (10.6)	—	—	—	
	6	12.7 (11.1)	13.3 (10.9)	—	—	—	
	8	12.6 (10.5)	11.7 (9.8)	—	—	—	
Body mass index, kg/m^2	2	20.0 (2.9)	20.4 (3.2)	—	—	—	0.59‖
	4	20.2 (3.0)	20.8 (3.3)	—	—	—	
	6	20.4 (3.0)	21.0 (3.3)	—	—	—	
	8	20.7 (3.0)	21.2 (3.3)	—	—	—	
Mid-upper arm circumference, cm	2	24.6 (3.4)	25.1 (3.5)	—	—	−0.07 (−0.24 to 0.10)	0.04‖
	4	24.9 (3.5)	25.4 (3.5)	—	—	−0.03 (−0.20 to 0.15)	
	6	25.3 (3.5)	25.7 (3.5)	—	—	0.08 (−0.09 to 0.26)	
	8	25.7 (3.5)	26.1 (3.6)	—	—	0.25 (0.08 to 0.43)	
Triceps skin-fold thickness, mm	2	11.1 (6.6)	10.9 (6.1)	—	—	—	0.82‖
	4	11.4 (6.7)	11.3 (6.1)	—	—	—	
	6	11.8 (6.8)	11.7 (6.2)	—	—	—	
	8	12.2 (6.9)	11.9 (6.2)	—	—	—	

(Continued)

Table 2. (*Continued*)

	Time Point (*wk*)	Vitamin D (*n = 190*)	Placebo (*n = 200*)	Adjusted Hazard Ratio (95% CI)*	Adjusted Odds Ratio (95% CI)*	Adjusted Mean Difference (95% CI)*	*P* Value
Scapular skin-fold thickness, mm	2	10.5 (5.7)	10.7 (5.7)	—	—	—	0.57‖
	4	10.9 (5.8)	11.1 (5.8)	—	—	—	
	6	11.1 (5.9)	11.4 (5.8)	—	—	—	
	8	11.5 (6.0)	11.7 (5.9)	—	—	—	

Definition of abbreviations: 25(OH)D = 25-hydroxyvitamin D; CI = confidence interval; IQR = interquartile range.
Data presented as mean (SD) unless otherwise noted.
*Analysis of sputum outcomes adjusted for age, sex, presence/absence of cavitation on baseline chest radiograph, baseline sputum smear (<100 vs. ⩾100 acid-fast bacilli per high-power field), drug-sensitivity profile (multidrug-resistant vs. non–multidrug-resistant), current smoking (yes vs. no), and current alcohol use (yes vs. no); analysis of chest radiograph involvement also adjusted for number of zones involved at baseline. Adjusted effect estimates for individual time points are only reported where *P* values for the relevant allocation–time interaction are <0.05.
†This analysis excludes participants who died, withdrew, or were lost to follow-up without having undergone sputum culture conversion.
‡This analysis also classifies the following as "nonconverters": participants who died before culture conversion (n = 4), and participants lost to follow-up before culture conversion (n = 20).
§Values for time to smear conversion >56 d cannot be estimated, as follow-up was truncated at 56 d; such values are therefore marked ">56."
‖*P* value for allocation–time interaction, used to evaluate an effect of allocation across all time points; values <0.05 indicate an effect of vitamin D supplementation on the outcome in question.

with historical reports indicating that vitamin D significantly improved clinical outcomes in the preantibiotic era (1), we conclude that favorable immunomodulatory effects of vitamin D supplementation are eclipsed by the relative potency of intensive-phase antimicrobial therapy in the context of drug-sensitive disease. Vitamin D might yet have a role in the prevention of *Mycobacterium tuberculosis* infection and/or disease (16): this hypothesis is being addressed by ongoing phase 3 clinical trials (17–19).

Might subgroups of patients derive a benefit from high-dose adjunctive vitamin D? Among patients with MDR TB, 5 of 13 patients receiving adjunctive vitamin D experienced sputum culture conversion at 8 weeks, as compared with 0 of 8 such patients randomized to adjunctive placebo ($P = 0.11$; Fisher exact test). This finding echoes the trend toward improved outcomes with adjunctive vitamin D in patients with MDR TB reported by Tukvadze and colleagues (7). Neither trial was individually powered to explore the effects of adjunctive vitamin D in patients with MDR TB, but the hypothesis that a host-directed therapy may be more efficacious in the context of disease that is less responsive to antimicrobials is plausible. Taken together, these findings suggest that a trial of adjunctive vitamin D in patients with MDR TB may be justified.

Another possibility is that subgroups of patients with particular mutations in genes in the vitamin D pathway may have enhanced responsiveness to adjunctive vitamin D. We have previously reported that patients homozygous for the minor allele of the TaqI VDR polymorphism had enhanced responsiveness to vitamin D (4). This finding was not replicated in the current study, possibly due to lack of power associated with the relatively low frequency of the minor allele of this polymorphism in Mongolia versus the study population in London (10.6 vs. 31.3%, respectively). We did, however, identify two other SNPs in VDR (rs4334089 and rs11568820) and—for the first time—one SNP in *CYP27B1* (rs4646536) that significantly modified the effect of vitamin D on sputum culture conversion after correction for multiple comparisons. *CYP27B1* governs conversion of 25(OH)D to the active metabolite 1,25-dihydroxyvitamin D, whereas VDR is the cognate receptor for this metabolite that mediates its influence on expression of more than 200 genes (20). It is therefore biologically plausible that polymorphisms in these two genes could modify effects of vitamin D supplementation—a hypothesis strengthened by reports of associations between these polymorphisms and diverse health outcomes (8).

Our study has several strengths. Time to sputum culture conversion was the primary outcome: this is a recognized surrogate for treatment failure and relapse (21). We were well-powered to detect a relatively modest effect of adjunctive vitamin D therapy: the study population had very low vitamin D status at baseline; administration of study medication was directly observed, assuring excellent adherence; and the regimen used in the active arm of the trial effectively elevated serum 25(OH)D concentrations into the high physiological range. Our genetic analysis was comprehensive, in that it explored the influence of variation in a total of 11 different genes in the vitamin D pathway; previous studies testing for genetic effect modification have only investigated polymorphisms in VDR (4, 7).

Our study also has some limitations. It was not powered to detect subgroup effects: null results of some subgroup analyses may therefore be attributable to type 2 error. We used an intermittent bolus dosing regimen to optimize adherence and correct vitamin D deficiency rapidly. There is some evidence to suggest that daily or weekly regimens of vitamin D supplementation may be optimal in enhancing host responses to respiratory pathogens (22); however, our finding that leukocyte counts differed between arms at follow-up indicates that the dosing regimen was effective in modulating the host response.

In conclusion, this large study used a high dose of vitamin D in a population with very low baseline vitamin D status but found no effect of this intervention on time to sputum culture conversion in the study population as a whole. Taken together with null results from other trials in the field for the outcome of sputum culture conversion, it seems unlikely that further such trials in patients with drug-sensitive disease will yield positive results. It is possible that the intervention may provide benefit in particular subgroups, such as those with MDR TB and individuals who have particular mutations in genes encoding the vitamin D receptor and the 25(OH)D 1α-hydroxylase enzyme; meta-analysis of individual participant data from these trials is ongoing (23), which will provide enhanced power to test these hypotheses. ■

Table 3. Time to Sputum Culture Conversion by Allocation and Subgroup

		n/N*	HR (95% CI)†	HR ratio (95% CI)‡	$P_{interaction}$‡
Clinical subgroups					
Baseline serum 25(OH)D concentration	<10 nmol/L	100/127	1.13 (0.75–1.68)	Ref.	0.92
	⩾10 nmol/L	194/257	1.06 (0.79–1.40)	0.98 (0.59–1.60)	
Sex	Male	190/256	1.25 (0.94–1.67)	1.41 (0.88–2.27)	0.16
	Female	108/134	0.89 (0.61–1.30)	Ref.	
Drug-resistance profile	Multidrug resistant	5/21	—§	—‖	—‖
	Other	293/369	1.06 (0.84–1.33)	Ref.	
Age	⩽33 yr	155/196	1.01 (0.73–1.38)	Ref.	0.28
	>33 yr	143/194	1.17 (0.83–1.64)	1.29 (0.81–2.06)	
Baseline cavitation	Absent	152/195	1.11 (0.81–1.53)	Ref.	0.81
	Present	146/195	1.05 (0.75–1.46)	0.95 (0.60–1.49)	
Baseline smear	<100 AFB per 100 high-power fields	92/112	1.07 (0.70–1.64)	Ref.	0.87
	⩾100 AFB per 100 high-power fields	206/278	1.12 (0.85–1.47)	1.04 (0.63–1.72)	
Completed trial per protocol¶	Yes	284/352	1.06 (0.84–1.34)	Ref.	0.11
	No	14/38	2.65 (1.33–5.28)	2.50 (0.81–7.72)	
Genetic subgroups					
Gene, SNP	Genotype				
VDR, rs4334089	GG	167/215	0.84 (0.62–1.14)	Ref.	0.003**
	GA/AA	128/171	1.71 (1.18–2.47)	2.03 (1.27–3.25)	
VDR, rs11568820 (Cdx2)	CC	160/205	0.84 (0.61–1.15)	Ref.	0.006**
	TC/TT	134/180	1.63 (1.13–2.34)	1.95 (1.21–3.12)	
VDR, rs9409929	GG	222/293	1.20 (0.91–1.56)	Ref.	0.22
	GA/AA	72/92	1.05 (0.62–1.79)	0.72 (0.42–1.22)	
VDR, rs10783219	TT	101/130	0.96 (0.65–1.44)	Ref	0.54
	TA/AA	190/252	1.14 (0.85–1.53)	1.17 (0.72–1.90)	
VDR, rs4516035	TT	250/324	1.10 (0.86–1.42)	Ref.	0.99
	TC/CC	43/60	1.37 (0.69–2.75)	1.01 (0.52–1.94)	
VDR, rs2238136	CC	143/192	1.22 (0.87–1.72)	Ref.	0.20
	TC/TT	148/190	0.93 (0.67–1.29)	0.74 (0.46–1.18)	
VDR, rs1544410	CC	229/302	1.08 (0.83–1.41)	Ref.	0.90
	TC	65/83	1.08 (0.64–1.81)	1.04 (0.59–1.82)	
VDR, rs2228570	GG	129/174	0.96 (0.67–1.37)	Ref.	0.58
	GA/AA	164/210	1.12 (0.82–1.54)	1.14 (0.71–1.82)	
VDR, rs2853559	GG	121/164	1.02 (0.71–1.46)	Ref.	0.75
	GA/AA	169/217	1.16 (0.85–1.58)	1.08 (0.67–1.73)	
VDR, rs7975232	CC	120/158	1.07 (0.74–1.56)	Ref.	0.93
	CA/AA	173/226	1.06 (0.78–1.44)	1.02 (0.64–1.64)	
VDR, rs7970314	AA	128/165	0.94 (0.66–1.34)	Ref	0.32
	GA/GG	164/218	1.22 (0.88–1.69)	1.27 (0.79–2.03)	
VDR, rs731236 (TaqI)	AA	236/310	1.06 (0.82–1.38)	Ref.	0.69
	GA/GG	58/75	1.08 (0.63–1.87)	1.13 (0.63–2.03)	
CYP27B1, rs4646536	GG	140/175	0.80 (0.56–1.14)	Ref.	0.02**
	GA/AA	152/208	1.47 (1.05–2.04)	1.73 (1.08–2.78)	
CYP27B1, rs4646537	TT	283/371	1.05 (0.83–1.34)	Ref.	0.04
	TG	11/14	19.43 (1.11–339.06)	3.71 (1.06–12.90)	
CYP24A1, rs6013897	TT	202/270	0.94 (0.71–1.24)	Ref.	0.04
	TA/AA	90/113	1.75 (1.12–2.73)	1.70 (1.02–2.81)	
CYP24A1, rs2762939	GG	128/168	1.01 (0.70–1.47)	Ref.	0.59
	GC/CC	166/217	1.17 (0.86–1.60)	1.14 (0.71–1.82)	
CYP24A1, rs2248137	CC	90/121	1.11 (0.72–1.71)	Ref	0.77
	GC/GG	201/261	1.10 (0.83–1.46)	0.93 (0.56–1.54)	
CYP24A1, rs2762934	GG	239/312	1.10 (0.85–1.43)	Ref.	0.96
	GA/AA	56/74	1.18 (0.67–2.08)	1.02 (0.58–1.85)	
CYP24A1, rs6127118	GG	129/170	0.96 (0.67–1.38)	Ref.	0.51
	GA/AA	158/206	1.22 (0.88–1.69)	1.18 (0.73–1.89)	
CYP27A1, rs17470271	AA	223/296	1.11 (0.85–1.45)	Ref.	0.92
	TA/TT	70/88	1.24 (0.74–2.07)	0.97 (0.57–1.67)	
CYP2R1, rs10500804	TT	110/145	1.38 (0.94–2.03)	Ref.	0.11
	TG/GG	183/239	0.93 (0.69–1.26)	0.68 (0.42–1.09)	

(Continued)

Table 3. (*Continued*)

		n/N*	HR (95% CI)†	HR ratio (95% CI)‡	$P_{interaction}$‡
CYP2R1, rs2060793	GG	109/146	0.91 (0.61–1.37)	Ref.	0.17
	AG/AA	179/233	1.23 (0.91–1.66)	1.41 (0.87–2.30)	
CYP2R1, rs10766197	GG	106/142	1.36 (0.92–2.01)	Ref.	0.16
	GA/AA	188/243	0.95 (0.70–1.28)	0.71 (0.44–1.15)	
CYP3A4, rs2740574	GG	292/382	1.07 (0.84–1.35)	—‖	—‖
	AG	2/3	—‖	—‖	
DBP, rs7041	AA	145/194	0.95 (0.67–1.33)	Ref.	0.38
	CA/CC	148/190	1.39 (0.99–1.95)	1.24 (0.77–1.97)	
DBP, rs12512631	TT	162/206	1.08 (0.78–1.49)	Ref.	0.61
	TC/CC	131/178	1.24 (0.86–1.77)	1.13 (0.71–1.81)	
DBP, rs4588	GG	174/227	1.27 (0.94–1.72)	Ref	0.30
	TG/TT	120/158	0.94 (0.65–1.36)	0.78 (0.49–1.25)	
DBP, rs2070741	GG	243/327	1.06 (0.82–1.37)	Ref.	0.83
	TG/TT	50/57	1.23 (0.67–2.27)	1.07 (0.58–1.99)	
DBP, rs2298849	AA	169/226	1.06 (0.78–1.45)	Ref.	0.90
	GA/GG	124/158	1.09 (0.76–1.57)	0.97 (0.61–1.55)	
DBP, rs16846876	AA	148/192	1.40 (0.99–1.96)	Ref.	0.22
	TA/TT	147/194	0.91 (0.65–1.28)	0.74 (0.47–1.19)	
DHCR7, rs3829251	GG	163/212	1.15 (0.84–1.57)	Ref.	0.75
	GA/AA	151/173	1.05 (0.73–1.51)	0.93 (0.58–1.48)	
DHCR7, rs12785878	GG	92/117	1.22 (0.80–1.86)	Ref.	0.50
	GT/TT	201/267	1.03 (0.78–1.38)	0.84 (0.51–1.39)	
LRP2, rs3755166	GG	100/127	1.07 (0.70–1.63)	Ref.	0.77
	GA/AA	195/259	1.13 (0.84–1.50)	1.08 (0.66–1.76)	
CUBN, rs3740165	GG	248/323	1.11 (0.86–1.44)	Ref.	0.66
	AG/AA	45/61	0.90 (0.46–1.77)	0.86 (0.45–1.66)	
RXRA, rs7861779	TT	280/367	1.12 (0.88–1.42)	Ref.	0.61
	CT/CC	15/19	0.97 (0.23–4.10)	0.76 (0.27–2.17)	
LTA4H, rs17525495	TT	164/217	1.04 (0.76–1.42)	Ref.	0.80
	TC/CC	130/168	1.20 (0.84–1.72)	1.06 (0.67–1.69)	

Definition of abbreviations: 25(OH)D = 25-hydroxyvitamin D; AFB = acid-fast bacilli; CI = confidence interval; *CUBN* = cubulin; *CYP* = cytochrome p450; *DBP* = vitamin D–binding protein; *DHCR7* = dehydrocholesterol reductase; HR = hazard ratio; *LRP2* = low density lipoprotein receptor–related protein-2; *LTA4H* = leukotriene A4 hydrolase; MDR = multidrug resistant; Ref. = reference; *RXRA* = retinoid X receptor α; SNP = single-nucleotide polymorphism; TB = tuberculosis; *VDR* = vitamin D receptor.

*N represents the number of subjects in a given subgroup, and n represents the number of participants within that subgroup who experienced sputum culture conversion during the trial.

†Hazard ratio from Cox regression, adjusting for age, sex, presence/absence of cavitation on baseline chest radiograph, baseline sputum smear (<100 vs. ⩾100 AFB per 100 high-powered fields), drug-sensitivity profile (MDR vs. non-MDR), current smoking (yes vs. no), and current alcohol use (yes vs. no).

‡From Cox regression using the whole sample and including an interaction between subgroup and allocation, adjusting for age, sex, presence/absence of cavitation on baseline chest radiograph, baseline sputum smear (<100 vs. ⩾100 AFB per 100 high-powered fields), drug-sensitivity profile (MDR vs. non-MDR), current smoking (yes vs. no), and current alcohol use (yes vs. no).

§The HR for effect of allocation on time to sputum culture conversion in participants with MDR TB could not be calculated, because 0 of 8 participants in this subgroup who were allocated to placebo experienced sputum culture conversion during the trial.

‖The HR ratio and corresponding *P* value could not be calculated because the HR for effect of allocation within the MDR subgroup could not be calculated (*see* previous footnote).

¶Per-protocol completion defined as completing trial having taken all doses of study medication.

**These *P* values remained significant when controlling the false discovery rate at 20% using a Benjamini-Hochberg procedure.

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