High-dose vitamin D_3 during intensive phase treatment of pulmonary tuberculosis: a double-blind randomised controlled trial

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Abstract

Background

Vitamin D was used to treat tuberculosis in the pre-antibiotic era, and its metabolites induce antimycobacterial immunity *in vitro*. Clinical trials investigating the effect of adjunctive vitamin D on sputum culture conversion are lacking.

Methods

We conducted a multi-centre randomised controlled trial of adjunctive vitamin D in adults with sputum smear-positive pulmonary tuberculosis in London, UK. 146 participants were allocated to receive 2.5 mg vitamin D_3 or placebo at baseline and at 2, 4 and 6 weeks after starting standard tuberculosis treatment. The primary endpoint of the trial was time from initiation of antimicrobial therapy to sputum culture conversion. Participants were genotyped for *Taq*I and *Fok*I polymorphisms of the vitamin D receptor (VDR), and interaction analyses were conducted to determine the influence of VDR genotype on response to vitamin D. This trial is registered with ClinicalTrials.gov (NCT00419068).

Findings

126 participants were included in the primary efficacy analysis (62 allocated to intervention, 64 allocated to placebo). Median time to sputum culture conversion was 36.0 days in the intervention arm and 43.5 days in the placebo arm (adjusted HR 1.39; 95% CI 0.90-2.16, P=0.14). *Taq*I genotype modified the effect of vitamin D supplementation on time to sputum culture conversion ($P_{interaction}$=0.03), with enhanced response seen only in participants with the *tt* genotype (HR 8.09, 95% CI

1.36-48.01, P=0.02). *Fok*I genotype did not modify the effect of vitamin D supplementation ($P_{interaction}$=0.85). Mean serum 25-hydroxyvitamin D at 8 weeks was 101.4 nmol/L vs. 22.8 nmol/L in intervention vs. placebo arms (95% CI for difference 68.6-88.2 nmol/L, P<0.001).

Interpretation

Administration of four doses of 2.5 mg vitamin D_3 elevated serum 25-hydroxyvitamin D concentrations in patients receiving intensive phase treatment for pulmonary tuberculosis and reduced time to sputum culture conversion in participants with the *tt* genotype of the *Taq*I VDR polymorphism.

Introduction

Tuberculosis (TB) is a major health problem: the World Health Organisation estimates that there were 9.4 million incident cases of TB in 2008, and 1.8 million deaths due to TB worldwide in 2008 (1). Augmentation of the immune response to *Mycobacterium tuberculosis* (MTB) has the potential to allow shortening of antimicrobial therapy in drug-sensitive disease, or to improve outcome in drug-resistant disease (2). Calcitriol, the active metabolite of vitamin D, induces antimycobacterial activity *in vitro* (3). It modulates the host response to mycobacterial infection by induction of reactive nitrogen and oxygen intermediates (4, 5), suppression of matrix metalloproteinase enzymes implicated in the pathogenesis of pulmonary cavitation (6) and induction of the antimicrobial peptide cathelicidin (7, 8) which induces autophagy (9). Calcitriol modulates immune responses by binding vitamin D receptor (VDR) expressed by antigen presenting cells and activated lymphocytes to regulate transcription of vitamin D-responsive genes. Human VDR is polymorphic: carriage of the *t* allele of the *Taq*I VDR polymorphism associates with enhanced calcitriol-induced phagocytosis of MTB *in vitro* (10) and more rapid sputum culture conversion in patients with pulmonary TB (PTB) (11). By contrast, carriage of the *f* allele of the *Fok*I VDR polymorphism associates with reduced transcriptional activity (12), reduced calcitriol-induced phagocytosis (10) and slower sputum culture conversion in PTB (11).

Numerous case series have reported that daily doses of 625 μg to 2.5 mg vitamin D enhance response to antimicrobial therapy for PTB (13). Randomised controlled trials investigating doses of vitamin D $\leq$ 125 μg/day or equivalent in active TB have shown no clinical benefit (14-17), but one trial investigating a higher dose regimen

(250 μg/day) did report reduced time to sputum smear conversion in the intervention arm (18). Sputum smear conversion is of limited value as a biomarker of treatment response in PTB however, because microscopy is less sensitive and less specific than culture for the detection of viable MTB bacilli in the sputum (19). Consequently multivariable analysis reveals that sputum culture conversion, but not sputum smear conversion, is an independent correlate of risk of long-term treatment failure or relapse (20). We therefore conducted a clinical trial to determine the effect of high-dose vitamin D on time to sputum culture conversion in patients receiving intensive phase antimicrobial therapy for PTB; sub-group analyses were performed to investigate whether the effect of adjunctive vitamin D on time to sputum culture conversion was modified by *Taq*I or *Fok*I VDR genotype.

Abbreviated Methods (full methods appear in Supplementary Material)

Participants

Study participants were recruited at 10 National Health Service Trusts in London, UK. Patients with newly-diagnosed PTB and acid-fast bacilli (AFB) visible on sputum smear microscopy were assessed for eligibility to participate. Full eligibility criteria are listed in Supplementary Material; principal exclusion criteria were age less than 18 years and serum corrected calcium >2.65 mmol/L. The study was approved by East London and The City Research Ethics Committee (ref 06/Q0605/83), and written informed consent was obtained from all participants before enrolment.

Procedures

Participants were randomly assigned to receive either four oral doses of 2.5 mg vitamin D_3 (Vigantol® oil, Merck Serono, Darmstadt, Germany) or organoleptically identical placebo (Miglyol® oil, Caesar and Loretz, Hilden, Germany) with allocation ratio 1:1. We have previously demonstrated that an oral dose of 2.5 mg vitamin D elevates serum 25-hydroxyvitamin D (25[OH]D) > 75 nmol/L in TB patients at one week post-administration (21). Randomisation was stratified according to presence/absence of cavitation on baseline chest radiograph. The first dose of study medication was administered within 7 days of starting antimicrobial treatment, and subsequent doses were administered at 2, 4 and 6 weeks after the start of antimicrobial treatment. Treatment allocation was concealed from participants and

study staff. All participants received intensive phase antimicrobial therapy comprising isoniazid, rifampicin, pyrazinamide and ethambutol in addition to study medication.

Participants completed a baseline clinical assessment including chest radiography, measurement of height and weight and collection of a sputum sample for microscopy and culture, a urine sample for determination of urinary calcium:creatinine ratio and a blood sample for determination of full blood count (FBC), 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 antimicrobial therapy; at each timepoint an overnight sputum specimen was collected from all patients who were able to expectorate spontaneously, weight was measured and urine and blood samples were collected as above. Chest radiography was repeated at 8 weeks.

Sputum specimens were examined for the presence of AFB by microscopy and inoculated into MB/BacT bottles (Organon Teknika, Boxtel, The Netherlands) which were placed in an incubator for monitoring until they signalled positive. The number of days from inoculation to positive signal for mycobacteria (days-to-positivity, DTP) was recorded for each uncontaminated sample. An inverse relationship between colony forming units (CFU) / ml of inoculum vs. DTP was demonstrated by inoculating MB/BacT bottles with 10-fold serial dilutions of a suspension of MTB H37Rv (Supplementary Figure S1). Additionally, at baseline and 8 weeks, samples were inoculated onto Löwenstein-Jensen slopes (Media for Mycobacteria, Penarth, UK). Concentrations of 25(OH)D were determined by isotope-dilution liquid

chromatography–tandem mass spectrometry (22). Genotyping was performed using Taqman allelic discrimination assays (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

The primary endpoint of the study was time from initiation of antimicrobial therapy to sputum culture conversion, estimated as the mid-point between the last positive sputum culture in broth and the first negative sputum culture in broth thereafter. Assuming a median time to sputum culture conversion of 5 weeks in the control group (23), follow-up of 8-weeks and accrual time of 2.5 years we calculated that 61 patients in each group would need to be recruited in order to detect a 2-week difference in median time to culture conversion between groups with 80% power using a 2-sided test at the 5% significance level (24). This number was increased by 20% to compensate for loss to follow-up, giving a total sample size of 146. Time to sputum smear conversion was a secondary endpoint, as were rate of rise in DTP, proportion of participants with negative sputum culture on solid medium at 8 weeks, change in serum corrected calcium concentration and urinary calcium:creatinine ratio during the study and 8-week 25(OH)D concentration, full blood count parameters, radiographic response to treatment and BMI. The primary safety endpoint was incidence of hypercalcaemia (serum corrected calcium >2.65 mmol/L). Efficacy analysis was by modified intention-to-treat, and excluded participants whose baseline sputum culture was negative or grew non-tuberculous mycobacteria. All participants who took at least one dose of study medication were included in the safety analysis.

Analyses were performed using SPSS (version 16.0, 2007), STATA (version 10.1, 2008) and R (version 2.8.1, 2009) software packages. Significance was tested at the

5% level. The primary analysis for effect of allocation on time to sputum culture conversion was conducted using a logrank test stratified according to presence/absence of cavitation on baseline chest radiograph. A multivariable Cox proportional hazards regression model stratified according to presence/absence of cavitation on baseline chest radiograph was obtained by fitting additional potential predictors of time to sputum culture conversion in turn. Time to sputum smear conversion was similarly analysed. Proportional hazards assumptions for Cox regression models were checked using Grambsch and Therneau's methods (25). A single pre-specified interim efficacy analysis of time to sputum culture conversion was performed after enrolment of 73 participants. The influence of allocation on categorical and continuous outcome measures compared at 8 weeks was evaluated using χ^2 tests and analysis of covariance respectively. Analysis of the effect of allocation on rate of rise of DTP was conducted using a linear mixed effects model; longitudinal changes in urinary calcium:creatinine ratio and serum corrected calcium were analysed using generalised estimating equations (GEE) with the assumption of an unstructured correlation matrix (26). Median age among randomised participants vs. potentially eligible individuals who declined randomisation was compared using a Mann Whitney test.

Role of the funding source

The British Lung Foundation was not involved in the design, conduct or analysis of the study, and did not review or approve the manuscript. Merck Serono donated Vigantol® and Miglyol® oil for the trial but did not influence study design, execution

or analysis. The decision to publish was made solely by the authors, and the corresponding author had full access to all data in the study.

Results

We assessed 464 patients for eligibility to participate in the trial between 25th January 2007 and 3rd July 2009: 214 were ineligible, 104 were eligible but declined randomisation and 146 were randomised. Reasons for ineligibility are presented in Table S1, Supplementary Material. There was no significant difference in median age between randomised participants vs. potentially eligible individuals who declined randomisation (31.2 years vs. 29.1 years respectively, P=0.07), but females were slightly under-represented among randomised participants vs. those declining randomisation (35/146 vs. 39/104 female respectively, P=0.02). The trial ended on the date of the final study visit of the last participant to be randomised. MTB complex was cultured from baseline sputum samples of 135 participants: follow-up sputum culture results were available for 126 participants (62 allocated to vitamin D, 64 allocated to placebo) who entered the primary efficacy analysis (Figure 1). Clinical and demographic characteristics of these participants were comparable for intervention vs. control group at baseline (Table 1). The majority of these participants were profoundly vitamin D deficient (serum 25(OH)D<20 nmol/L in 75/126) and almost all were vitamin D insufficient (serum 25(OH)D<75 nmol/L in 122/126) at baseline.

The median time to sputum culture conversion was 36 days (95% CI 31.8-40.2 days) in the intervention arm and 43.5 days (95% CI 36.5-50.5 days) in the control arm (Figure 2; unadjusted P=0.41, logrank test). White/Latin American ethnic origin, presence of >3 AFBs per high-power field on baseline sputum smear, baseline neutrophil count $\geq 7.5 \times 10^6$/ml and older age were associated with delayed sputum culture conversion on univariate analysis (table 2); the hazard ratio (HR) for effect of

allocation after adjustment for these factors in the Cox regression model was 1.39 (95% CI 0.90-2.16, P=0.14). The effect of allocation on time to sputum culture conversion was significantly modified by *Taq*I genotype ($P_{interaction}$=0.03): vitamin D significantly hastened sputum culture conversion in participants with the *tt* genotype (HR 8.09, 95% CI 1.36-48.01, P=0.02), but not in those with *Tt* genotype (HR 0.85, 95% CI 0.45-1.63, P=0.63) or *TT* genotype (HR 1.13, 95% CI 0.60-2.10, P=0.71). There was no evidence that participants' *Fok*I genotype modified the effect of adjunctive vitamin D on time to sputum culture conversion however ($P_{interaction}$=0.85). Non-statistically significant trends towards faster rise in DTP in the intervention arm (9% faster rise; 95% CI -23%-43%, P=0.57; Fig S2) and faster sputum smear conversion in the intervention arm (adjusted HR 1.26, 95% CI 0.83-1.93, P=0.28; Fig S3) were also seen. The proportion of participants with negative sputum culture on solid medium at 8 weeks was similar in intervention and control arms of the study (41/52 vs. 45/56 respectively, P=0.85).

Mean serum 25(OH)D concentration at 8 weeks was 101.4 nmol/L in the intervention arm of the study vs. 22.8 nmol/L in the control arm (95% CI for difference 68.6-88.2 nmol/L, P<0.001; Fig 3A). Allocation to the intervention arm of the study also induced an increase in urinary calcium:creatinine ratio from the 4-week timepoint onwards (P<0.001, GEE; Fig 3B). Mean serum corrected calcium fell in both arms over the course of the study (P<0.001, GEE), but the rate of fall was not influenced by allocation (P=0.67, GEE; Fig 3C). Allocation to vitamin D was associated with an increase in lymphocyte count and a borderline significant decrease in monocyte count (Table 3), such that mean lymphocyte: monocyte ratio was significantly higher in the intervention vs. control arm of the study at 8 weeks (3.52 vs. 2.70, 95% CI for

difference 0.16-1.11, P=0.009). No statistically significant effect of allocation on body mass index, extent of CXR involvement, CRP or ESR was observed at 8 weeks (Table 3).

Two patients died during the study, one in each arm; neither death was attributed to study medication (Table 4). Eight serious adverse events (SAE) were observed in 7 out of the 71 participants who received at least one dose of vitamin D; two SAE were observed in 2 out of the 70 participants who received at least one dose of placebo. Study medication was discontinued in three participants in the intervention arm of the study who experienced adverse events (AE) that were judged to be potentially related to vitamin D by physicians blinded to allocation. One of these participants experienced a paradoxical upgrading reaction (enlarging paraspinal abscess) and mild hypercalcaemia (corrected calcium 2.68 mmol/L) after receiving two doses of 2.5 mg vitamin D; one experienced a paradoxical upgrading reaction (enlarging psoas abscess) without hypercalcaemia after receiving two doses of 2.5 mg vitamin D; and one experienced asymptomatic mild hypercalcaemia (corrected calcium 2.72 mmol/L) after receiving 2 doses of 2.5 mg vitamin D. Participants with paradoxical upgrading reaction underwent drainage of collections and made a good recovery; corrected serum calcium normalised within 2 weeks of discontinuing vitamin D in both participants who experienced hypercalcaemia. The frequency of all other AEs was similar between arms with the exception of symptoms of upper respiratory tract infection, which were reported by 1/71 patients receiving ≥ 1dose of vitamin D vs. 6/70 patients receiving ≥1dose of placebo.

Discussion

We report that administration of four doses of 2.5 mg vitamin D_3 to patients receiving intensive phase treatment for smear-positive PTB significantly hastened sputum culture conversion in a sub-group of participants with the *tt* genotype of the *Taq*I vitamin D receptor polymorphism. A large increase in mean serum 25(OH)D concentration was observed among participants in the intervention arm of the trial.

Our study has several strengths. Participants in the intervention arm of the trial received a preparation with verified vitamin D_3 content at a dose sufficient to increase mean serum 25(OH)D concentration >100 nmol/L, while those in the placebo arm of the trial were almost universally vitamin D insufficient throughout the study; the very high prevalence of vitamin D deficiency among TB patients in London has previously been reported, and has been attributed to lack of sunshine at latitude 51°N(21). We determined the effect of adjunctive vitamin D on sputum culture conversion - the only phase II TB trial outcome measure shown to correlate with long-term risk of relapse (27). We also demonstrated an inverse relationship between MTB CFU/ml and DTP in liquid medium, and investigated the effect of vitamin D supplementation on the speed of elimination of bacilli from sputum, as estimated by rate of rise of DTP in liquid culture of serial sputum specimens. This represents an important methodological advance, and builds on the work of the Oflotub Consortium who have utilised non-linear mixed effects modelling of serial sputum colony counts to assess sterilising activity of fluoroquinolones (28). All sputum samples in our study were analysed in a single laboratory that was close to study sites, ensuring standardisation of culture and microscopy methods and minimising loss of bacillary viability in

transit. Additionally, we investigated the effect of adjunctive vitamin D on numbers of different circulating leucocyte populations, and observed an increase in lymphocyte: monocyte ratio in participants in the intervention arm of the study - a biomarker of disease resolution in animal models of TB (29). Finally, we explored the possibility that the effect of vitamin D supplementation might be modified by VDR genotype, and found that individuals with *tt* genotype of the *Taq*I polymorphism have enhanced responsiveness to vitamin D supplementation. The hypothesis that carriage of the *t* allele for this synonymous single nucleotide polymorphism might associate with increased VDR mRNA stability (30) has been refuted by two subsequent studies demonstrating no influence of the *Taq*I polymorphism on mRNA stability (31, 32); the mechanism by which this gene: environment interaction is mediated remains to be determined.

Our study has some limitations. Serum 25(OH)D concentration > 75 nmol/L was not attained in all participants in the intervention arm at 8 weeks, suggesting that some participants may not have taken all doses of study medication. Moreover, the 8-week duration of the study did not allow follow-up after completion of intensive phase therapy; this precluded analysis of the effect of adjunctive vitamin D on risk of relapse post-treatment.

Randomised trials have not previously investigated the effects of adjunctive vitamin D on sputum culture conversion in TB patients, to our knowledge, but trials using other primary outcome measures have been performed. The largest of these (17) reported that administration of three doses of 2.5 mg vitamin D_3 at baseline, 5 and 8 months, did not influence clinical severity score or time to sputum smear conversion

in 365 patients in Guinea Bissau; however in that trial, participants' mean baseline 25(OH)D was >70 nmol/L, and the dosing regimen utilised was too low to influence serum 25(OH)D concentrations at follow-up. A smaller trial conducted in 67 patients in Indonesia (18) reported enhanced sputum smear conversion at 6 weeks, but not at 8 weeks, after initiation of antimicrobial treatment in patients receiving 250 μg vitamin D daily. The clinical significance of this finding is hard to interpret as sputum smear conversion, unlike sputum culture conversion, is not an independent correlate with long-term risk of treatment failure or relapse (20). Moreover, vitamin D content of study medication used in this study was not verified, and serum 25(OH)D concentrations in participants in this study were not reported before or after supplementation.

The incidence of hypercalcaemia in our study is much lower than that seen by Narang *et al*, who reported hypercalcaemia in 19/30 patients with smear positive pulmonary TB taking a daily dose of 10-95 μg vitamin D (15). The actual dose of vitamin D administered in the Narang study has been called into question, however (33); if it was significantly higher than reported, this would explain the discordance in reported incidence of hypercalcaemia between that study and our own. We also found that serum corrected calcium declined in both intervention and control groups following initiation of antimicrobial therapy. This may have occurred due to a reduction in granulomatous burden in patients responding to therapy, resulting in a decrease in extra-renal 1-alpha hydroxylation of 25(OH)D and a fall in serum 1,25-dihydroxyvitamin D concentrations. Paradoxical upgrading reactions were seen in two participants in the intervention arm vs. none in the control arm of our study. We discontinued study medication in these patients as a precaution, as some case series

have reported an association between paradoxical upgrading reaction and administration of vitamin D (13). The incidence of paradoxical upgrading reaction in the intervention arm of our study (3%) was considerably lower than the 10% previously reported in HIV uninfected patients (34), and a causal relationship between vitamin D supplementation and paradoxical upgrading reaction is not demonstrated. The observation that symptoms of upper respiratory tract infection (URTI) were markedly less frequent in the intervention arm of our study is in keeping with that reported by Aloia *et al* who observed a reduction in URTI symptoms among vitamin D-supplemented participants in an osteoporosis trial (35).

In conclusion, we report that administration of four oral doses of 2.5 mg vitamin D corrected deficiency safely and quickly in patients receiving intensive phase therapy for pulmonary TB, but did not significantly enhance response to therapy in the study population as a whole. However, vitamin D supplementation did significantly reduce time to sputum culture in conversion among participants with the *tt* genotype of the *Taq*I VDR polymorphism, suggesting that this sub-group of TB patients may derive clinical benefit from vitamin D supplementation. Investigation of the mechanism by which this gene: environment interaction is mediated is warranted.

Contributors

ARM, CJG, GHB, RND, NJW, RJW and PMT contributed to study design. ARM, YH, KI, CJG, GHB, GEP, JCM-G, MD, RND, HJM, LVB, and RDB participated in implementation of the trial. ARM, YH, KI and CJG participated in administration and regulatory support, data management and data collection. PMT, APC, FAD, VN, TRV, KEB, CJM and AKC developed and performed assays of biochemical and microbiological outcome measures. CAM developed and performed genotyping assays. NJW read chest radiographs. ARM, CMR, SME and GRD contributed to data analysis. ARM wrote the manuscript; all other authors critically reviewed it and approved the final version.

Conflict of interest statement

Merck Serono donated €7,000 to Queen Mary, University of London, to support an academic meeting entitled 'Vitamin D: mechanisms of action in health and disease'; this meeting was convened by ARM and CJG. All other authors declare that they have no conflict of interest.

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Figure legends

Figure 1: Trial Profile

Figure 2: Time to sputum culture conversion by allocation. Error bars, 95% confidence intervals. The number of participants with positive sputum culture remaining in follow-up (number at risk) at 0, 2, 4, 6 and 8 weeks is presented (36).

Figure 3: A. Serum 25-hydroxyvitamin D concentration by allocation at baseline and 8 weeks. B. Mean urinary calcium: creatinine molar ratio by allocation at baseline and 2, 4, 6 and 8 weeks. Error bars, standard errors of the mean. C. Mean serum corrected calcium by allocation at baseline and 2, 4, 6 and 8 weeks. Error bars, standard errors of the mean.

Table 1. Baseline characteristics by allocation

	Vitamin D	**N**	**Placebo**	**N**
Median age, years (IQR)	30.7 (24.5 to 41.5)	62	30.5 (24.8 to 38.4)	64
Female	14 (23%)	62	14 (22%)	64
Ethnic group		62		64
- Asian / Asian British	30 (48%)		25 (39%)	
- Black / Black British	17 (27%)		26 (41%)	
- White / Latin American	15 (24%)		13 (20%)	
Occupation		62		64
- Student	10 (16%)		9 (14%)	
- Employed	46 (74%)		49 (77%)	
- Unemployed	6 (10%)		6 (9%)	
HIV status		62		64
- Sero-positive	3 (5%)		2 (3%)	
- Sero-negative	43 (69%)		39 (61%)	
- Undetermined	16 (26%)		23 (36%)	
Diabetes mellitus	3 (5%)	62	2 (3%)	64
Median duration of symptoms pre-diagnosis, months (IQR)	2.0 (1.5 to 4.0)	62	2.0 (1.0 to 3.0)	64
Median duration of treatment pre-enrolment, days (IQR)	2.0 (0.0 to 4.0)	62	2.0 (0.0 to 3.8)	64
Administration of antimicrobial treatment		62		64
- Daily, self-administered	61 (98%)		62 (97%)	
- 3 x weekly, directly-observed	1 (2%)		2 (3%)	
Baseline sputum smear		62		64
- ≤3 AFB per high-power field	27 (44%)		31 (48%)	
- >3 AFB per high-power field	35 (57%)		33 (52%)	
Rifampicin-resistant isolate	0 (0%)	62	4 (6%)	64
Serum 25(OH)D, nmol/L	21.1 (20.0)	62	21.3 (19.0)	64
Serum 25(OH)D <20 nmol/L	36 (58%)	62	39 (61%)	64
Serum 25(OH)D <75 nmol/L	59 (95%)	62	63 (98%)	64
Serum corrected calcium, mmol/L	2.45 (0.08)	62	2.45 (0.09)	64
Urinary calcium:creatinine ratio	0.34 (0.28)	59	0.37 (0.26)	58
Haemoglobin, g/dL	12.6 (1.9)	62	12.6 (1.8)	64
Mean corpuscular volume, fl	82.7 (7.2)	59	82.7 (7.1)	61
Total white blood cell count x 10^9/L	9.2 (3.1)	62	8.1 (2.6)	64
Neutrophil count x 10^9/L	6.8 (2.8)	62	5.7 (2.3)	64
Lymphocyte count x 10^9/L	1.4 (0.6)	62	1.4 (0.5)	64
Monocyte count x 10^9/L	0.8 (0.4)	62	0.8 (0.3)	64
Platelet count x 10^9/L	409 (136)	62	381 (149)	64
Erythrocyte sedimentation rate, mm/h	62.1 (23.1)	57	60.9 (17.4)	60
C reactive protein, mg/L	71.4 (49.5)	62	60.5 (45.0)	62
Body mass index, kg/m^2	20.1 (3.1)	60	20.2 (2.7)	64
Baseline chest radiograph				
- Cavities present	36 (58%)	62	36 (56%)	64
- Zones affected	2.8 (1.3)	47	2.8 (1.3)	49
*Taq*I vitamin D receptor genotype		62		64
- TT	30 (48%)		29 (45%)	
- Tt	27 (44%)		28 (44%)	
- tt	5 (8%)		7 (11%)	
*Fok*I vitamin D receptor genotype		62		64
- FF	29 (47%)		27 (42%)	
- Ff	25 (40%)		24 (38%)	
- ff	8 (13%)		13 (20%)	

Data are number (%) or mean (standard deviation) except where stated. IQR, inter-quartile range.

Table 2: Cox regression analysis, time to sputum culture conversion

			Univariate analysis		**Multivariable analysis[1]**	
		N	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**
Allocation	Active	62	1.18 (0.77 to 1.80)	0.46	1.39 (0.90 to 2.16)	0.14
	Placebo	64	ref	-	ref	-
Age, yr		126	0.97 (0.95 to 0.99)	0.006	0.97 (0.95 to 1.00)	0.02
Sex	Female	28	1.23 (0.75 to 2.02)	0.41	-	-
	Male	98	ref	-	-	-
Ethnic group	Asian / Asian British	55	2.84 (1.45 to 5.59)	0.002	2.80 (1.40 to 5.62)	0.004
	Black / Black British	43	2.66 (1.34 to 5.26)	0.005	2.65 (1.33 to 5.27)	0.005
	White / Latin American	28	ref	-	ref	-
Baseline chest radiograph	Cavitation present	72	0.97 (0.64 to 1.49)	0.89	1.15 (0.73 to 1.80)	0.54
	Cavitation absent	54	ref	-	ref	-
Baseline sputum smear	≤3 AFB per HPF	58	2.03 (1.31 to 3.15)	0.002	1.85 (1.16 to 2.97)	0.01
	>3 AFB per HPF	68	ref	-	ref	-
Baseline neutrophil count	<7.5 x 10^6/ml	92	1.97 (1.18 to 3.29)	0.01	1.88 (1.09 to 3.25)	0.02
	≥7.5x10^6/ml	34	ref	-	ref	-
Baseline serum 25(OH)D	<20 nmol/L	75	1.27 (0.82 to 1.96)	0.28	-	-
	≥20 nmol/L	51	ref	-	-	-
TaqI genotype	TT	59	1.30 (0.61 to 2.78)	0.50	-	-
	Tt	55	1.22 (0.57 to 2.62)	0.62	-	-
	tt	12	ref	-	-	-
FokI genotype	FF	56	1.00 (0.57 to 1.78)	0.99	-	-
	Ff	49	0.66 (0.36 to 1.22)	0.19	-	-
	ff	21	ref	-	-	-

Ref, reference category. AFB, acid-fast bacilli. HPF, high power field. 1. Adjusted for age, ethnicity, baseline sputum smear, neutrophil count and presence / absence of cavitation on baseline chest radiograph

Table 3: Secondary outcome measures at 8 weeks, by allocation

	Vitamin D	**Placebo**	**95% CI for difference**	**P**
Haemoglobin, g/dL	13.27 (1.64)	13.35 (1.54)	-0.49 to 0.31	0.67
Mean corpuscular volume, fl	85.45 (7.25)	83.78 (6.99)	0.06 to 2.59	0.04
Total white blood cell count x 10^9/L	7.32 (2.71)	6.82 (2.11)	-0.70 to 0.84	0.85
Lymphocyte count x 10^9/L	1.80 (0.71)	1.60 (0.60)	0.03 to 0.40	0.02
Monocyte count x 10^9/L	0.57 (0.26)	0.66 (0.25)	-0.17 to 0.01	0.09
Neutrophil count x 10^9/L	4.61 (2.34)	4.24 (1.94)	-0.78 to 0.65	0.86
Platelet count x 10^9/L	304 (104)	294 (105)	-30 to 37	0.83
Erythrocyte sedimentation rate, mm/hr	29.90 (25.91)	30.11 (25.22)	-9.27 to 9.76	0.96
C reactive protein, mg/L	19.59 (25.23)	17.82 (22.73)	-7.14 to 9.71	0.76
Body mass index	21.29 (2.72)	21.18 (2.75)	-0.28 to 0.46	0.63
Chest radiograph: zones affected	2.30 (1.29)	2.28 (1.18)	-0.45 to 0.27	0.62

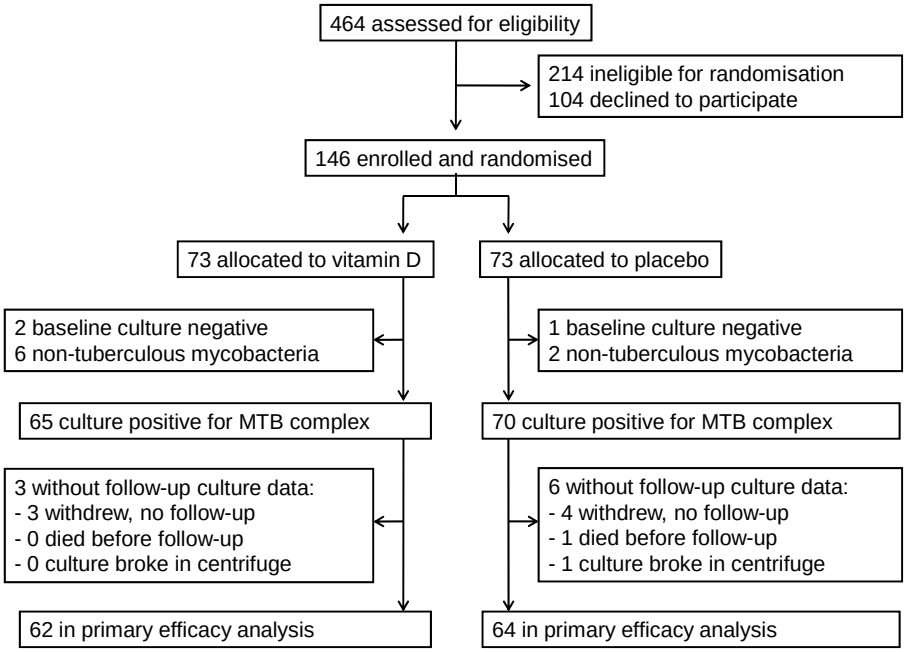
Data are mean (standard deviation)

Table 4. Adverse events

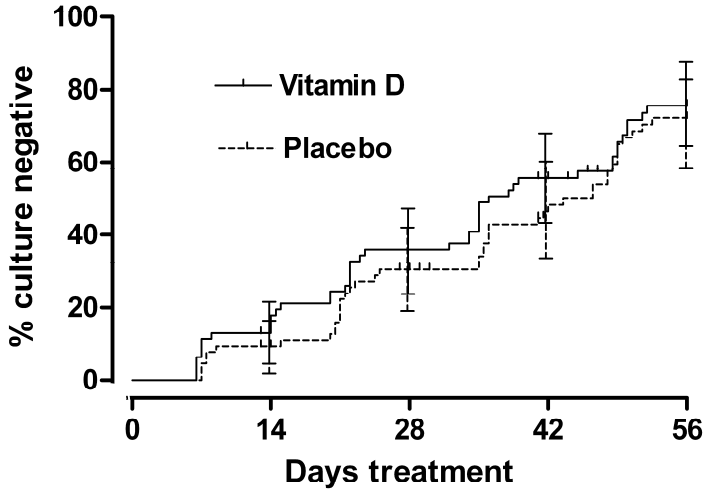
		Vitamin D (n=71)	Placebo (n=70)
Serious adverse events[1]			
	Multi-organ failure secondary to alcoholic liver disease[2]	0	1
	Lobar pneumonia[2]	1	0
	Paradoxical upgrading reaction	2	0
	Nausea / vomiting	1	0
	Epistaxis	1	0
	Rifampicin-induced haemolytic anaemia[3]	1	0
	Bowel perforation[3]	1	0
	COPD exacerbation	1	0
	Non-compliance with medication	0	1
	Number experiencing any serious adverse event	7	2
Non-serious adverse events			
	Nausea / vomiting	9	7
	Pruritis	5	5
	Arthralgia	4	7
	Hepatitis	4	1
	Upper respiratory tract infection	1	6
	Hypercalcaemia	2	0
	Hypocalcaemia	5	2
	Urinary calcium:creatinine molar ratio ≥ 1	16	9
	Other non-serious adverse event	21	21
	Number experiencing any non-serious adverse event	37	32

1. Adverse events were classified as serious if they caused death or were life-threatening, or if they required hospitalisation or prolongation of existing hospitalisation
2. These adverse events were fatal
3. Both adverse events were experienced by the same participant

Martineau *et al*, Figure 1



Martineau *et al*, Figure 2



Number at risk					
Vitamin D	62	53	39	26	12
Placebo	64	57	42	29	15

Martineau*et al*, Figure 3

