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Adjunctive vitamin D in tuberculosis treatment: meta-analysis of individual participant data

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ABSTRACT

Background: Randomised controlled trials (RCTs) of adjunctive vitamin D in pulmonary tuberculosis (PTB) treatment have yielded conflicting results. Individual participant data (IPD) meta-analysis could identify factors explaining this variation.

Methods: We meta-analyzed IPD from RCTs of vitamin D in patients receiving antimicrobial therapy for PTB. Primary outcome was time to sputum culture conversion. Secondary outcomes were time to sputum smear conversion, mean 8-week weight and incidence of adverse events. Pre-specified sub-group analyses were done according to baseline vitamin D status, age, sex, drug-susceptibility, HIV status, extent of disease, and vitamin D receptor genotype.

Results: IPD were obtained for 1,850 participants in 8 studies. Vitamin D did not influence time to sputum culture conversion overall (aHR 1.06, 95% CI 0.91-1.23), but it did accelerate sputum culture conversion in participants with multidrug-resistant PTB (aHR 13.44, 95% CI 2.96-60.90); no such effect was seen in those whose isolate was sensitive to rifampicin and/or isoniazid (aHR 1.02, 95% CI 0.88-1.19; $P_{interaction}$ =0.02). Vitamin D accelerated sputum smear conversion overall (aHR 1.15, 95% CI 1.01-1.31), but did not influence other secondary outcomes.

Conclusions: Vitamin D did not influence time to sputum culture conversion overall, but it accelerated sputum culture conversion in patients with multidrug-resistant PTB.

INTRODUCTION

The World Health Organisation estimates that 10.0 million people developed active tuberculosis in 2017, and that 1.6 million people died of this disease. [1] Existing antimicrobial treatment for active tuberculosis requires lengthy administration of a high pill burden that carries a significant risk of toxicity and has limited efficacy in drug-resistant disease. Adjunctive host-directed therapies (i.e. immunomodulatory therapies given in addition to antimicrobial treatment) have the potential to reduce duration of transmissibility, shorten antimicrobial therapy and improve outcomes in drug-resistant disease.[2] Vitamin D has attracted interest as a potential candidate on the basis of its historical use in tuberculosis treatment,[3] reported associations between vitamin D deficiency and susceptibility to tuberculosis infection and disease,[4,5] and its recognized role in supporting antimycobacterial immune responses.[6,7] Double-blind randomized placebo-controlled trials evaluating effects of vitamin D supplementation on sputum culture and/or smear conversion in patients receiving antimicrobial therapy for pulmonary tuberculosis have yielded conflicting results: four report favorable effects on their primary outcome in the study population as a whole,[8-11] two report benefits on their primary outcome in sub-groups only,[12,13] and four report no effects of the intervention on their primary outcome.[14-17] Systematic reviews and aggregate data meta-analyses including at least some of these studies[18-21] are all limited by lack of access to individual participant data, which precludes conduct of sub-group analyses to explore whether individual-level factors may modify responses to vitamin D supplementation. This is an important omission, because uneven distribution of such effect-modifiers between participants in different settings might underlie variation in results seen between trials.

For example, effects of vitamin D supplementation may be modified by drug-susceptibility of a patient's *Mycobacterium tuberculosis* isolate: this might be expected on the grounds that individuals with drug-resistant disease may stand to derive particular benefit from host-directed therapy where antimicrobial therapies are less effective.[2] Alternatively, effects of vitamin D supplementation may be stronger in, or restricted to, individuals with lower baseline vitamin D status, since these individuals might be expected to derive the greatest benefit from vitamin D replacement. This phenomenon has been demonstrated in other respiratory diseases.[22-24] We therefore set out to obtain individual participant data from the ten clinical trials listed above and meta-analyze them in order to obtain an updated estimate of overall efficacy, and to determine whether effects of this intervention vary according to the presence or absence of potential effect-modifiers.

METHODS

Protocol and Registration

The methods for this systematic review and meta-analysis were described in an outline protocol that was registered with the PROSPERO International Prospective Register of Systematic Reviews, number CRD42015020288 (http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42015020288). Research ethics committee approval to contribute individual participant data from completed primary trials to the current meta-analysis was not required for any study. Findings are reported according to the PRISMA guidelines for individual participant data meta-analysis.[25]

Selection of studies and participants

Double-blind, placebo-controlled randomized controlled trials of adjunctive vitamin D supplementation including patients receiving standard antimicrobial treatment for pulmonary tuberculosis were eligible for inclusion if the primary trial had been approved by a Research Ethics Committee, and if data on sputum culture and/or smear conversion were reported. Studies in which vitamin D was given in combination with another intervention were excluded if effects of vitamin D could not be isolated (e.g. by use of a factorial design). Studies in which a factorial design was used to investigate effects of other therapies alongside vitamin D were included, as these allowed effects of vitamin D to be isolated.

Individual participants in eligible studies were excluded if pulmonary tuberculosis was not confirmed by sputum culture or smear, i.e. we only included data from individual patients in the meta-analysis if they had either a positive sputum smear at baseline or a positive sputum culture at baseline or both.

Data Sources and Searches

Two investigators (DAJ and ARM) searched PubMed, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL) and Web of Science using the electronic search strategies described in the Appendix. Searches were regularly updated up to and including 10th November 2017. No language restrictions were imposed. Two investigators (DAJ and ARM) determined which studies met the eligibility criteria.

Data Extraction and Quality Assurance

Individual participant data were requested from the Principal Investigator for each eligible trial, and the terms of collaboration were specified in a data transfer agreement, signed by representatives of the data provider and the recipient (Queen Mary University of London). Data were de-identified at source prior to transfer *via* email. On receipt, two investigators (DAJ and ARM) assessed data integrity by performing internal consistency checks and by cross-checking proportions of participants with positive 8-week sputum culture and smear by arm against those reported in published papers. Study authors were contacted to provide missing data and to resolve queries arising from these

integrity checks. Once queries had been resolved, clean data were uploaded to the main study database, which was held in STATA IC v12 (College Station, TX, USA).

The following data relating to study characteristics were collected: study setting, eligibility criteria, details of adjunctive therapies being evaluated in each trial and duration of follow-up. Individual participant data were extracted for the following variables relating to baseline characteristics: age, sex, weight, circulating 25(OH)D concentration, drug susceptibility, human immunodeficiency virus (HIV) status, extent of disease on baseline chest radiograph (as measured by the proportion of zones involved and the presence or absence of cavitation), genotype for single nucleotide polymorphisms (SNPs) in the gene encoding the vitamin D receptor (*VDR*, *Fok*I and *Taq*I), and study allocation (vitamin D *vs* placebo). Follow-up data were requested for time from initiation of antibiotic treatment to stable sputum culture/smear conversion (the date of stable culture/smear conversion being estimated as the midpoint between the date of the last positive sputum culture/smear and the date of the first negative sputum culture/smear thereafter); sputum culture and smear status, weight and circulating 25(OH)D concentration after 8 weeks of antimicrobial treatment; and occurrence of serious adverse events, death, study withdrawals, and potential adverse reactions to vitamin D supplementation (hypercalcemia or renal stones).

Risk of Bias Assessment for Individual Studies

We used the Cochrane Collaboration Risk of Bias tool[26] to assess the following variables: sequence generation, allocation concealment, blinding of participants,

personnel and outcome assessors, completeness of outcome data, evidence of selective outcome reporting and other potential threats to validity. Selectivity of reporting was assessed either by comparing study protocols against study reports, or by specifically asking study authors whether all pre-specified outcomes were reported. Study quality was assessed independently by two investigators (ARM and DAJ), except for the trials in which ARM was an investigator,[12,13] which were assessed by RR and MAH. Discrepancies were resolved by consensus.

Definition of Outcomes

The primary outcome of the meta-analysis was time from initiation of antimicrobial therapy to stable sputum culture conversion, estimated as the midpoint between the last positive sputum culture and the first negative sputum culture thereafter. This outcome was selected as primary because it is a recognized surrogate for treatment failure and relapse that reflects the longitudinal profile of culture results over time, as opposed to a single time point.[27] Participants who were unable to expectorate spontaneously at follow-up time points were deemed to be culture negative. Where participants were culture positive at 8-week follow-up (or earlier if lost to follow-up before this time point), 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 time to stable sputum smear conversion, estimated as the midpoint between the last positive sputum smear and the first negative sputum smear thereafter; proportions of participants with negative sputum culture / smear after 8 weeks of antimicrobial therapy; weight after 8 weeks of antimicrobial

therapy; and risk of death, study withdrawals, and potential adverse reactions to vitamin D supplementation (hypercalcemia or renal stones). Time to sputum culture / smear conversion was estimated only for trials where sputum was collected at intervals of two weeks or less, due to the potential for imprecision of such an estimate with less frequent sputum collection.

Data Synthesis and Analysis

DAJ and ARM analyzed the data using STATA IC, version 12. Our individual participant data meta-analysis approach followed published guidelines.[28] We did both one-step and two-step individual participant data meta-analyses for the primary outcome of time to sputum culture conversion. For secondary outcomes, we did one-step individual participant data meta-analysis only. In the one-step approach, individual participant data from all studies were modelled simultaneously while accounting for the clustering of participants within studies. We used mixed models, with a random effect for study and fixed effects for age and sex (reflecting an assumption that the influence of age and sex on response to treatment would not vary across trials) to obtain the pooled intervention effect with a 95% CI. We did not adjust for other covariates because missing values for some participants would have led to their exclusion from statistical analyses. We analyzed survival data using mixed-effects parametric survival models; effects of the intervention on time to sputum culture/smear conversion were expressed as adjusted hazard ratios (aHRs). Survival data for time to sputum culture and smear conversion were truncated at 8 weeks in order to capture effects of adjunctive vitamin D on the standardized background of intensive-phase antimicrobial therapy. We analyzed

proportions of participants experiencing an event using mixed-effects logistic regression; effects of the intervention on dichotomous outcomes were expressed as adjusted odds ratios (aORs). In the two-step approach, individual participant data were first analyzed for each separate study independently to produce a hazard ratio for the effect of allocation on time to sputum culture conversion for that study, adjusted for age and sex. We then calculated a weighted average of adjusted hazard ratios for each study and summarized heterogeneity using the I^2 statistic.

Exploration of Variation in Effects

To identify factors modifying the effects of vitamin D supplementation, we did pre-specified subgroup analyses for the primary outcome of time to sputum culture conversion by extending the one-step meta-analysis framework to include treatment-covariate interaction terms. Subgroups were defined according to baseline vitamin D status (circulating 25[OH]D concentration <25 *vs* ≥25 nmol/L), age (< *vs* ≥ the median value of 30 years), sex, drug susceptibility (multidrug-resistant [i.e. resistant to at least rifampicin and isoniazid] *vs* not), HIV status (seronegative *vs* seropositive), extent of chest radiograph involvement at baseline (<50% vs ≥50% of zones involved and presence *vs* absence of cavitation), vitamin D dosing regimen (daily/weekly *vs* less frequently), and VDR genotype for the *Fok*I and T*aq*I SNPs (analyzed using the per-allele method i.e. under an additive model). The 25 nmol/L cut-off for baseline 25(OH)D concentration in the subgroup analyses was selected because it is the threshold for vitamin D deficiency defined by the UK Department of Health,[29] and because we have previously shown this threshold to modify host response to respiratory infection.[22] The

Benjamini–Hochberg procedure for multiple testing correction was applied to the family of P values for interaction to control the false discovery rate (FDR) at 20%.

Risk of Bias Assessment Across Studies

For the primary analysis, the likelihood of publication bias was investigated through the construction of a contour-enhanced funnel plot.

Additional analyses

We did a pre-specified responder analysis in participants assigned to the intervention arm of included studies, comparing time to sputum culture conversion in participants who attained a circulating 25(OH)D concentration of 75 nmol/L or higher *vs* those who did not. We did two exploratory adjustments of the sub-group analysis evaluating effects of vitamin D in patients with vs. without MDR-TB, controlling for baseline vitamin D status (25[OH]D <25 nmol/L vs. ≥25 nmol/L) and presence vs. absence of cavitation on baseline chest radiograph in addition to the pre-specified adjustments for age, sex and clustering of participants within trials. A pre-specified sensitivity analysis excluding studies deemed at high risk of bias was not conducted, since no included trial fell into this category.

RESULTS

Study Selection and Individual Participant Data Obtained

The study selection process is illustrated in Figure 1. Our search identified 513 unique studies that were assessed for eligibility, of which 10 studies with a total of 2,223 randomized participants fulfilled eligibility criteria. Individual participant data were sought for all studies, and obtained for 8/10 studies (total 2,096 participants). Individual participant data were not obtained for 2 studies (total of 127 participants): in one case the corresponding author indicated that individual participant data were not available,[8] and in the other, the corresponding author did not respond to an invitation to contribute individual participant data to this meta-analysis.[11] A total of 246 randomized participants in the 8 studies for which individual participant data were obtained were excluded from the meta-analysis on the grounds that they did not have pulmonary tuberculosis confirmed by sputum culture and/or smear. All of the remaining 1,850 participants contributed data to analysis of serious adverse events; 1,163 participants contributed data to analysis of sputum culture conversion, and 1,611 participants contributed data to analysis of sputum smear conversion. Differences in the total number of participants contributing to these analyses represent differences in data availability: serious adverse event data were available for all, but microbiological outcome data were not.

Study and Participant Characteristics

The eight studies for which individual participant data were obtained were conducted in eight countries on three continents (Table 1). All studies investigated effects of vitamin

D_3; none investigated vitamin D_2. Six studies used two-arm parallel designs to investigate effects of vitamin D_3 only; [9,12-14,16,17] two studies investigated effects of other host-directed therapies concurrently using a factorial design.[10,15] Seven studies administered vitamin D_3 orally to participants in the intervention arm: this was given daily,[10] weekly then 2-weekly,[17] 2-weekly,[12,13,16] 4-weekly,[15] and three times over an 8-month period (at baseline, 5 months and 8 months).[14] One study administered vitamin D_3 intramuscularly to participants in the intervention arm at baseline and 4 weeks.[9] Duration of follow-up ranged from 8 weeks to 8 months. Primary outcomes included one or more of sputum culture conversion,[10,12,13,15-17] clinical scores,[10,14,15] weight gain[9] and end-study chest radiograph involvement.[9] Data relating to final treatment outcome were available for 331 participants in two trials[14,15] (Appendix Table 1). The 1,850 participants included in individual participant data meta-analysis ranged in age from 15 to 86 years, and 650 (35.1%) were female. Baseline HIV status was tested in 995 participants in 5 studies,[12,14-17] and found to be positive in 157 (15.8%) of them. Baseline 25(OH)D concentrations were determined in 1,660 participants in 6 studies, [10,12-14,16,17] and ranged from undetectable to 250 nmol/L: 688 participants tested (41.5%) had baseline 25(OH)D levels <25 nmol/L. Drug susceptibility testing results were available for 1,350 participants in 6 studies: 55 participants tested (4.1%) had multidrug-resistant tuberculosis (i.e. their *M. tuberculosis* complex isolate was resistant to isoniazid and rifampicin at least), of whom 39 (70.9%) commenced second-line anti-TB therapy during their participation in the trials; relatively early initiation of second-line therapy was made possible by the use of molecular methods in some studies that enabled the rapid detection of mutations associated with drug-resistance. Baseline characteristics of

participants were comparable between study arms for the study population as a whole (n=927 allocated to vitamin D *vs* n=923 allocated to placebo; Appendix Table 2) and for the sub-group of participants with multidrug-resistant tuberculosis (n=30 allocated to vitamin D *vs* n=25 allocated to placebo; Appendix Table 3). All studies included sputum smear conversion as an outcome measure; six studies additionally included sputum culture conversion as an outcome measure.[10,12,13,15-17] Individual participant data integrity checks did not reveal any discrepancies between primary reports *vs* individual participant data supplied.

Risk of Bias within Studies

Details of the risk of bias assessment are provided in Appendix Table 4. Four trials were assessed as being at unclear risk of bias due to the relatively high proportion (>20%) of participants who were lost to follow-up.[10,14-16]

Overall effect, primary outcome

Overall, vitamin D supplementation did not have a statistically significant effect on the primary outcome of time to sputum culture conversion after initiation of antimicrobial therapy, either in one-step analysis (adjusted hazard ratio [aHR] 1.06, 95% CI 0.91 to 1.23, P=0.44; 820 participants in 4 studies;[12,13,16,17] Figure 2A) or in two-step analysis (aHR 1.06, 95% CI 0.91 to 1.23, P=0.46; P for heterogeneity =0.84; 820 participants in 4 studies;[12,13,16,17] Figure 3).

Sub-group analyses, primary outcome

Sub-group analyses were conducted using one-step individual participant data meta-analysis to investigate whether effects of adjunctive vitamin D supplementation on time to sputum culture conversion differed according to baseline vitamin D status, age, sex, drug susceptibility, HIV status, extent of disease on baseline chest radiograph, presence or absence of cavitation on baseline chest radiograph, type of dosing regimen and *VDR* genotype. Results are presented in Table 2. Vitamin D supplementation accelerated sputum culture conversion among individuals with multi-drug resistant pulmonary tuberculosis (aHR 13.44, 95% CI 2.96 to 60.90; 37 participants in 4 studies; [12,13,16,17] within sub-group P=0.001) but not in those whose isolate was sensitive to rifampicin and/or isoniazid (aHR 1.02, 95% CI 0.88 to 1.19; 780 participants in 4 studies;[12,13,16,17] within sub-group P=0.32; P for interaction =0.02). The P value for interaction for this sub-group analysis remained significant after correction for multiple comparisons testing, using the Benjamini and Hochberg method with a false discovery rate of 20%. Because we noted a degree of imbalance in the proportion of patients with MDR TB who had cavitation in intervention vs. control arms (26.7% vs. 48.0% respectively, Appendix Table 3) we conducted an exploratory sub-group analysis additionally controlling for presence vs. absence of cavitation. Results of the subgroup analysis were not materially affected (within-subgroup aHR for patients with MDR TB 11.43, 95% CI 2.22 to 58.94; 36 participants in 3 studies;[12,13,17] within sub-group P=0.004; P for interaction = 0.03). We also noted that the prevalence of baseline vitamin D deficiency was higher in participants with vs. without MDR TB (61.8% vs. 41.4% respectively, P=0.003). However, results of the sub-group analysis were not

materially affected when additional adjustment was made for the presence vs. the absence of vitamin D deficiency at baseline (P for interaction =0.02). P values for interaction for all other sub-group analyses were greater than 0.05.

Secondary outcomes, efficacy

Adjunctive vitamin D accelerated sputum smear conversion (median time to sputum smear conversion, 21 days for vitamin D vs. 26 days for placebo; aHR 1.15, 95% CI 1.01, 1.31, P=0.03; 1,200 participants in 6 studies;[10,12-16] Figure 2B). However, the proportion of participants with negative sputum culture and smear at 8 weeks was not significantly different for those randomized to vitamin D *vs* placebo (490/577 [84.9%] *vs* 484/586 [82.6%] culture negative at 8 weeks, aOR 1.17, 95% CI 0.85 to 1.60, P=0.33, 1,163 participants in 6 studies;[10,12,13,15-17] 644/806 [79.9%] *vs* 608/805 [75.5%] smear negative at 8 weeks, aOR 1.29, 95% CI 1.00 to 1.67, P=0.051; 1,611 participants in 8 studies). [9,10,12-17] Administration of adjunctive vitamin D did not influence mean weight after 8 weeks of antimicrobial therapy in participants randomized to vitamin D *vs* placebo (53.2 *vs* 53.4 kg, respectively; mean difference 0.13 kg, 95% CI -0.24 to 0.49 kg, P=0.43; 1,634 participants in 8 studies).[9,10,12-17]

Secondary outcomes, safety

Results of one-step individual participant data meta-analysis of safety outcomes are reported in Appendix Table 5. No participant experienced renal stones. There was no difference in the proportion of participants allocated to vitamin D vs. placebo experiencing hypercalcemia (6.2% vs. 6.1%, P=0.83), serious adverse events of any

cause (3.6% vs. 3.5%, P=0.96), study withdrawal (12.6% vs. 13.4%, P=0.79) or death due to any cause (2.5% vs. 2.3%, P=0.79).

Risk of Bias Across Studies

A funnel plot for the outcome of time to sputum culture conversion did not suggest publication bias in relation to this outcome, since randomized controlled trials showed even spread of results on both sides of the overall adjusted hazard ratio (Appendix Figure 1).

Responder Analysis

Results of responder analysis are presented in Appendix Table 6. Among participants randomized to the intervention arm of studies for which end-study 25(OH)D data were available, no difference in time to sputum culture conversion was observed between participants who attained a serum 25(OH)D ≥75 nmol/L *vs* those who did not.

DISCUSSION

We report results of the first meta-analysis of individual participant data from randomized controlled trials of adjunctive vitamin D in patients with pulmonary tuberculosis. In the study population as a whole, vitamin D supplementation did not influence the primary outcome of time to sputum culture conversion, but it did modestly accelerate sputum smear conversion, which was a secondary outcome. Pre-specified sub-group analysis revealed that vitamin D accelerated sputum culture conversion in participants with multidrug-resistant pulmonary tuberculosis, but not in those whose mycobacterial isolate was sensitive to rifampicin or isoniazid or both. Vitamin D supplementation was safe at the doses administered: no instances of renal stones were seen, and serious adverse events were evenly distributed between participants randomized to vitamin D *vs* placebo.

Our overall finding of no effect of vitamin D on time to sputum culture conversion is consistent with results from existing systematic reviews and aggregate data meta-analyses, although our findings of accelerated sputum smear conversion are at variance with them.[18-21] In contrast to the lack of effect overall, the favorable effect of vitamin D on time to culture conversion that we demonstrate in the sub-group of patients with multidrug-resistant tuberculosis is of potential clinical significance. Moreover, it is biologically plausible, since host-directed therapies such as vitamin D are likely to confer greater benefit in scenarios where antimicrobial therapy is less effective.[2] Caution in interpreting results of this sub-group analysis is warranted for several

reasons. First, a significant minority (29.1%) of participants with MDR TB did not receive any second-line antimicrobial therapy during their participation in the trials; thus, findings cannot be generalized to patients receiving optimal antimicrobial treatment for MDR TB. Second, the analysis is based on a relatively small number of participants – accordingly the 95% CI for the adjusted hazard ratio is wide. Third, the potential for a positive result arising by chance as a consequence of several sub-group analyses being conducted cannot be discounted. However, we minimized the potential for type 1 error by pre-specifying a limited number of sub-group analyses, each of which was supported by an independent hypothesis; the P value for interaction for this sub-group analysis (0.02) remained significant after correction for multiple comparisons testing. All in all, when taken together with data from observational studies showing that low 25(OH)D associates with delayed sputum smear conversion in patients receiving second-line therapy for multidrug-resistant pulmonary tuberculosis,[30] and considering the low cost and low toxicity of adjunctive vitamin D supplementation, the positive finding in this sub-group provides a rationale for conducting a new randomized controlled trial to investigate effects of this intervention in patients receiving second-line antimicrobial therapy for multidrug-resistant tuberculosis. However, in our judgement, the strength of evidence from this sub-group analysis is not sufficient to justify a clinical recommendation to use of adjunctive vitamin D in the treatment of MDR TB without such a new primary trial being conducted.

Strengths and limitations of this study

Our study has several strengths. It is unique in that we had access to individual participant data: this allowed us to conduct sub-group analyses to determine whether effects of vitamin D supplementation varied between individuals. We included data from eight out of ten eligible studies (88% of participants worldwide), none of which were assessed as being at high risk of bias, and restricted analysis to patients who had microbiologically confirmed tuberculosis. Included studies recruited patients from low- and high-incidence settings in three continents, enhancing generalizability of our results. Our primary outcome of time to sputum culture conversion is accepted as the preferred endpoint for phase II trials of new TB regimens.[27] Our definition captured stable culture conversion only, i.e. if a patient became culture negative but subsequently reverted to culture positive, this was not classified as sputum culture conversion.

Our study also has some limitations. We failed to obtain individual participant data from 2/10 eligible trials.[8,11] However, neither of these studies investigated our primary outcome of sputum culture conversion, and both were small (n=67 and n=60): their omission therefore has a limited impact on secondary outcomes only. Of note, both trials reported favorable effects of vitamin D on their primary outcome: therefore, if their omission does introduce a bias, that bias would likely be towards the null. Interpretation of the Funnel plot (Appendix Figure 1) is limited by the small number of studies included, but the fact that the smaller randomized controlled trials showed an equal spread of results on both sides of the overall adjusted hazard ratio provides some reassurance that publication bias was not a major issue in our meta-analysis. A further

limitation is that end-of-treatment outcomes were not available for meta-analysis: this reflects that fact that almost all trials had relatively short follow-up. However, in the absence of a consistent signal from the phase 2B studies included in this meta-analysis, phase 3 trials of adjunctive vitamin D with end-of-treatment outcomes are not likely to be conducted. Our meta-analysis therefore represents the best evidence in this field that is likely to become available.

Conclusions and policy implications

In conclusion, we show that adjunctive vitamin D does not influence time to sputum culture conversion in drug-sensitive pulmonary tuberculosis, but it may accelerate sputum culture conversion in patients with multidrug-resistant disease. Randomized controlled trials of vitamin D supplementation in patients with multidrug-resistant pulmonary tuberculosis are therefore justified.

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Author Contributions: ARM initiated the project and wrote the study protocol. DAJ and ARM assessed eligibility of studies for inclusion. DAJ, ARM, RR and MAH performed risk of bias assessments. DG, CW, RR, NS, PD, APR, TRZ and ARM were all directly involved in the acquisition and supply of individual participant data for the work. DAJ designed and executed statistical analyses, with input from ARM. ARM and DAJ wrote the first draft of the report. All authors revised it critically for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Competing Interests

All authors have completed the ICMJE uniform disclosure form. We did not receive specific financial support for this work. No author has had any financial relationship with any organizations that might have an interest in the submitted work in the previous three

years. No author has had any other relationship, or undertaken any activity, that could appear to have influenced the submitted work.

Transparency Declaration

ARM is the manuscript's guarantor and he affirms that the manuscript is an honest, accurate, and transparent account of the study being reported. The study was conducted as pre-specified in the protocol, except for the following aspects. Sub-group analysis of the primary outcome by vitamin D dose (<4,000 vs. ≥4,000 IU per day or equivalent) was not performed, since studies investigating doses of <4,000 IU per day or equivalent[14,15] did not evaluate time to sputum culture conversion. Sub-group analysis of the primary outcome by type of vitamin D administered (D_2 vs D_3) was not performed, since vitamin D_2 was not administered in any included study: all investigated vitamin D_3. A pre-specified sensitivity analysis excluding studies deemed at high risk of bias was not conducted, since no included trial fell into this category. In response to reviewers' requests we did two exploratory adjustments of the sub-group analysis evaluating effects of vitamin D in patients with vs. without MDR-TB in addition to the pre-specified adjustments for age, sex and clustering of participants within trials: one included additional adjustment for baseline vitamin D status (25[OH]D <25 nmol/L vs. ≥25 nmol/L) and the other included additional adjustment for presence vs. absence of cavitation on baseline chest radiograph.

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Table 1: Characteristics of trials and participants included in individual participant data meta-analysis

Study first author & year	Setting	Dose and route of vitamin D_3, intervention arm	Anti-TB therapy	N included / N randomized	N allocated to vitamin D: placebo	Mean age, years: included (s.d.) [range]	Male: Female	Proportion HIV-infected at baseline[1]	Proportion with MDR TB	25(OH)D assay	Mean baseline 25(OH)D, nmol/L (s.d.) [range]	Proportion with baseline 25(OH)D <25 nmol/L[1]	Duration of follow-up	Primary outcome	Sputum culture ascertainment	Sputum smear ascertainment
Wejse 2009 [14]	Guinea Bissau	2.5 mg at 0/5/8 months, oral	2 months HRZE, then 6 months HE	241/367[2]	117:124	36.1 (13.4) [16-80]	144:97	82/240 (34.2%)	Not tested	LC-MS/MS	77.7 (23.2) [35 – 191]	0/232 (0.0%)	8 months	Clinical score	Not done	0,2,4,6,8 weeks, 5, 8 months
Martineau 2011 [12]	UK	2.5 mg at 0/2/4/6 weeks, oral	2 months HRZE	135/146[3]	70:65	33.4 (11.6) [18-75]	103:32	5/93 (5.4%)	1/135 (0.7%)	LC-MS/MS	20.7 (19.1) [undetectable – 94]	96/135 (71.1%)	8 weeks	Sputum culture conversion	0,2,4,6,8 weeks	0,2,4,6,8 weeks
Salahuddin 2013 [9]	Pakistan	15 mg at 0/4 weeks, intramuscular	2 months HRZE, then 6 months HE	259/259	132:127	28.1 (13.6) [16-86]	141:118	Not tested	Not tested	ECLIA	54.2 (23.7) [10 – 135]	36/259 (13.9%)	12 weeks	Weight gain and chest radiograph involvement (co-primary)	Not done	0,4,8,12 weeks
Ralph 2013 [15]	Indonesia	1.25 mg at 0/4 weeks, oral (factorial design with L-arginine)	2 months HRZE then 4 months HR	164/200[4]	86:78	31.0 (11.2) [15-65]	109:55	19/123 (15.4%)	2/164 (1.2%)	N/A	Not measured	Not measured	24 weeks	Sputum culture conversion and clinical score (co-primary)	0,4,8 weeks	0,1,2,3,4, 5,6,7,8,12, 16,20,24 weeks
Mily 2015 [10]	Bangladesh	0.125 mg daily for 2 months, oral (factorial design with oral PBA)	2 months HRZE then 4 months HR	260/288[5]	132:128	27.2 (8.0) [18-59]	162:98	Not tested	7/260 (2.7%)	ECLIA	26.7 (16.3) [8 – 91]	142/256 (55.5%)	24 weeks	Sputum culture conversion and clinical score (co-primary)	0,4,8 weeks	0,1,2,3,4, 6,8,10,12, 24 weeks
Daley 2015 [16]	India	2.5 mg at 0/2/4/6 weeks, oral	2 months HRZE	209/247[6]	101:108	43.0 (14.8) [18-72]	162:47	0/209 (0%)	1/209 (0.5%)	ECLIA	63.0 (49.7) [10 – 250]	34/204 (16.7%)	8 weeks	Sputum culture conversion	0,2,4,6,8 weeks	0,2,4,6,8 weeks
Tukvadze 2015 [17]	Republic of Georgia	1.25 mg weekly for 8 weeks, then alternate weeks for 8 weeks, oral	2 months HRZE then 4 months HR	192/199[7]	97:95	33.5 (11.6) [18-63]	123:69	3/184 (1.6%)	23/192 (12.0%)	LC-MS/MS	37.4 (22.1) [6 – 104]	69/190 (36.3%)	16 weeks	Sputum culture conversion	0,2,4,6,8,12,16 weeks	0,8 weeks
Ganmaa 2017 [13]	Mongolia	3.5 mg at 0/2/4/6 weeks, oral	2 months HRZE	390/390	190:200	35.9 (13.9) [17-84]	256:134	Not tested	21/390 (5.4%)	CLIA	17.2 (24.2) [undetectable – 188]	311/384 (81.0%)	8 weeks	Sputum culture conversion	0,2,4,6,8 weeks	0,2,4,6,8 weeks

1. Where denominators are less than total number of included participants, data are missing
2. 126 excluded (113 smear negative at baseline, 11 extra-pulmonary tuberculosis only, 2 with final diagnosis other than tuberculosis)
3. 11 excluded (8 cultured non-tuberculous mycobacteria, 3 culture negative at baseline)
4. 36 excluded (19 culture negative at baseline, 13 culture missing at baseline, 4 culture contaminated at baseline)
5. 28 excluded (all culture negative at baseline)
6. 38 excluded (37 culture negative at baseline, 1 culture missing at baseline)

7. 7 excluded (all culture negative at baseline)

40,000 international units (IU) vitamin D_3 = 1 mg; 25(OH)D concentrations reported in ng/ml were converted to nmol/L by multiplying by 2.496. 25(OH)D, 25-hydroxyvitamin D; N/A, not applicable; LC-MS/MS, liquid chromatography tandem-mass spectrometry; ECLIA, electrochemiluminescence immunoassay; CLIA, chemiluminescence immunoassay; MDR TB, multidrug-resistant tuberculosis, i.e. resistant to isoniazid and rifampicin at least; PBA, 4-phenylbutyrate; H, isoniazid; R, rifampicin; Z, pyrazinamide; E, ethambutol.

Table 2: One-step individual participant data meta-analysis, time to sputum culture conversion after initiation of antimicrobial therapy: overall and by sub-group.

	N participants (N trials)	Median time to sputum culture conversion, placebo group (IQR)	Median time to sputum culture conversion, vitamin D group (IQR)	Adjusted hazard ratio (95% CI)[1]	P value	P value for Interaction
Overall	820 (4)	35 (21, 49)	35 (21, 49)	1.06 (0.91, 1.23)	0.44	
Baseline 25(OH)D concentration						
<25 nmol/L	480 (4)	35 (22, 49)	35 (21, 49)	1.14 (0.93, 1.40)	0.20	0.25
≥25 nmol/L	330 (4)	31 (20, 44)	34 (19, 46)	0.94 (0.75, 1.19)	0.62	
Age						
<30 years	350 (4)	35 (21, 49)	34 (20, 45)	1.09 (0.87, 1.37)	0.45	0.70
≥30 years	470 (4)	35 (23, 49)	35 (22, 49)	1.04 (0.85, 1.27)	0.74	
Sex						
Male	559 (4)	35 (22, 49)	35 (21, 49)	1.10 (0.91, 1.32)	0.32	0.53
Female	261 (4)	34 (21, 47)	34 (19, 44)	0.99 (0.77, 1.29)	0.97	
Multidrug-resistant isolate[2]						
No	780 (4)	35 (21, 49)	35 (21, 49)	1.02 (0.88, 1.19)	0.78	0.02[4]
Yes	37 (4)	>56 (50, >56)[3]	38 (10, >56)[3]	13.44 (2.96, 60.90)	0.001	
HIV status						
Seronegative	383 (3)	29 (20, 43)	29 (16, 41)	1.03 (0.84, 1.28)	0.75	0.53
Seropositive	8 (2)	21 (9, 36)	39 (10, 49)	0.09 (0.01, 0.89)	0.04	
% zones involved, baseline CXR						
<50%	377 (2)	35 (21, 50)	35 (21, 49)	--[5]	--[5]	0.75
≥50%	132 (2)	37 (28, 49)	36 (33, 50)	1.05 (0.70, 1.58)	0.82	
Cavitation, baseline CXR						
No	380 (3)	35 (21, 49)	35 (21, 45)	1.13 (0.90, 1.42)	0.29	0.60
Yes	294 (3)	36 (22, 50)	35 (21, 51)	1.03 (0.79, 1.34)	0.83	
Vitamin D Dosing Regimen						
Daily/weekly	165 (1)	23 (11, 37)	23 (9, 37)	--[5]	--[5]	0.68
Bolus/2-weekly	655 (3)	35 (21, 49)	35 (21, 49)	1.08 (0.91, 1.28)	0.39	
***Fok*I *VDR* genotype**						
FF	226 (2)	36 (23, 50)	35 (21, 51)	0.99 (0.73, 1.35)	0.97	0.81
Ff	221 (2)	36 (21, 50)	35 (34, 49)	1.20 (0.88, 1.64)	0.24	
ff	56 (2)	36 (35, 49)	49 (22, 50)	1.00 (0.55, 1.82)	0.99	
***Taq*I *VDR* genotype**						
TT	394 (3)	36 (22, 50)	35 (22, 50)	1.10 (0.88, 1.38)	0.41	0.95
Tt	162 (3)	36 (21, 49)	35 (21, 52)	0.96 (0.68, 1.37)	0.83	
tt	42 (3)	35 (22, 53)	34 (9, 40)	1.54 (0.77, 3.08)	0.22	

1, adjusted for age, sex and clustering within trials, except for sub-group analysis by age (where age was not adjusted for) and sex (where sex was not adjusted for) **2**, isolate resistant to both isoniazid and rifampicin, at least. **3**, survival times were truncated at 56 days in order to capture effects of adjunctive vitamin D on the standardized background of intensive-phase antimicrobial therapy; values for time to sputum culture conversion of greater than 56 days are therefore marked '>56'. **4,** the P value for interaction for this sub-group analysis remained significant after correction for multiple comparisons testing, using the Benjamini and Hochberg method with a false discovery rate of 20%. **5**, within-subgroup adjusted hazard ratio and P value could not be calculated due to non-convergence of model.

Figure 1: PRISMA Diagram of Study Selection

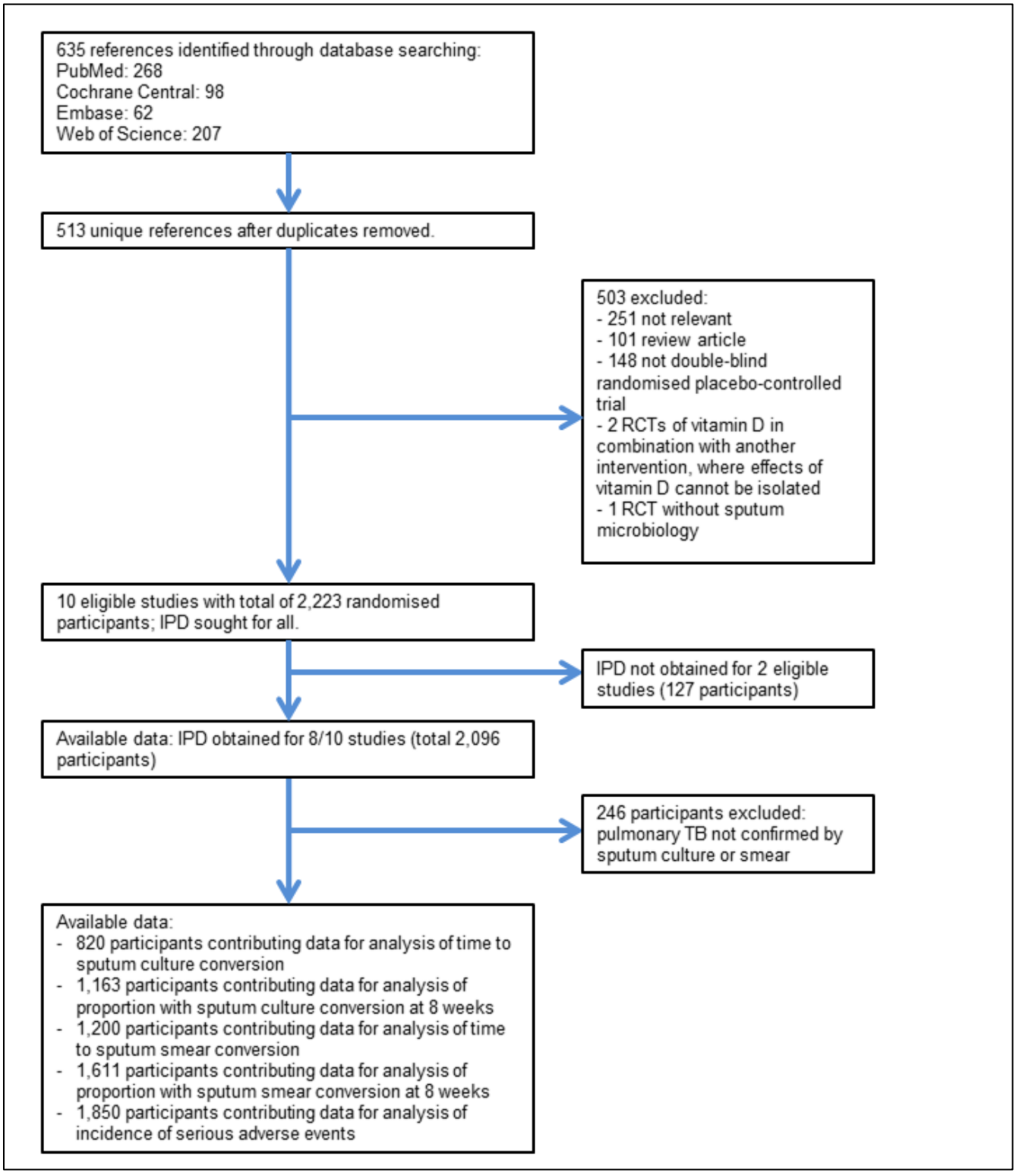
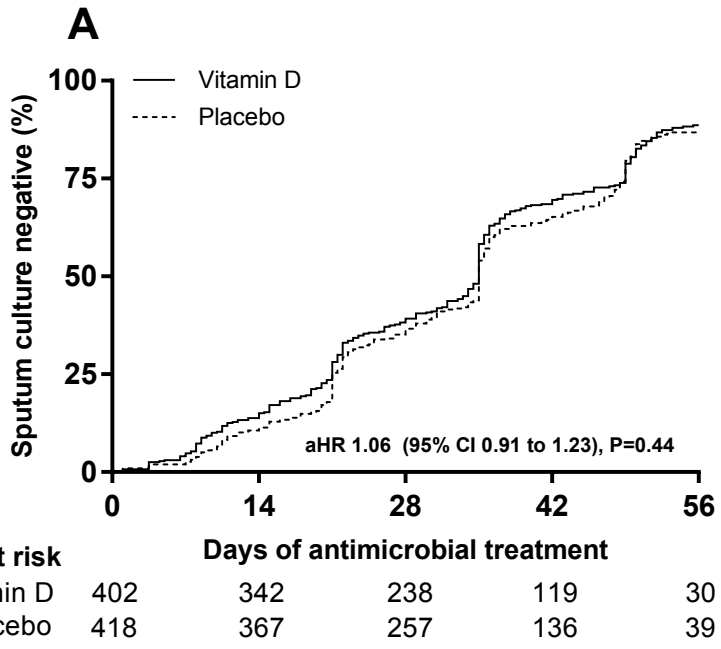


Figure 2: Time to sputum culture and smear conversion by allocation: one-step individual participant data meta-analysis. A, time to sputum culture conversion by allocation. **B**, time to sputum smear conversion by allocation. Numbers of participants with positive sputum culture or smear remaining in follow-up (number at risk) at 0, 14, 28, 42, and 56 days are shown. Adjusted hazard ratios (aHR) with 95% confidence intervals (CI) are from mixed models with a random effect for study and fixed effects for age and sex.



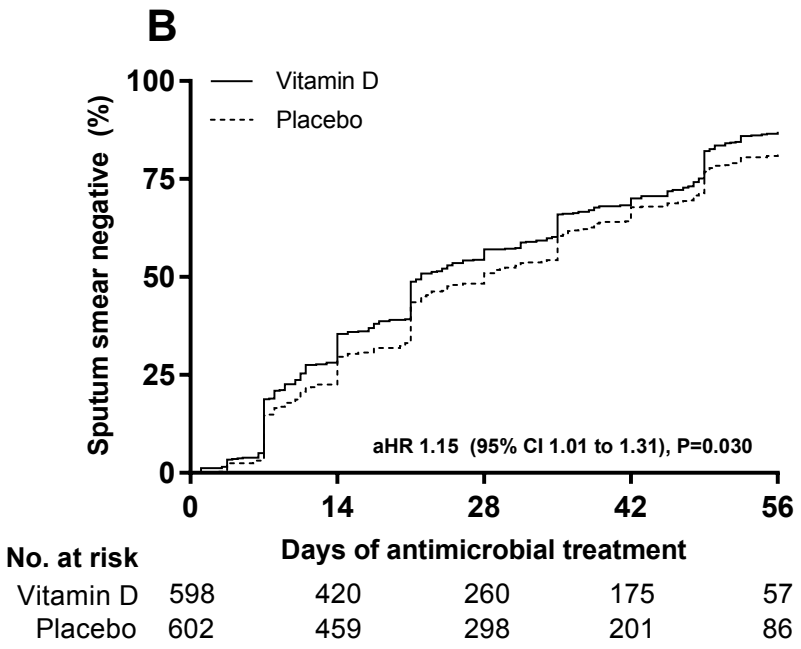
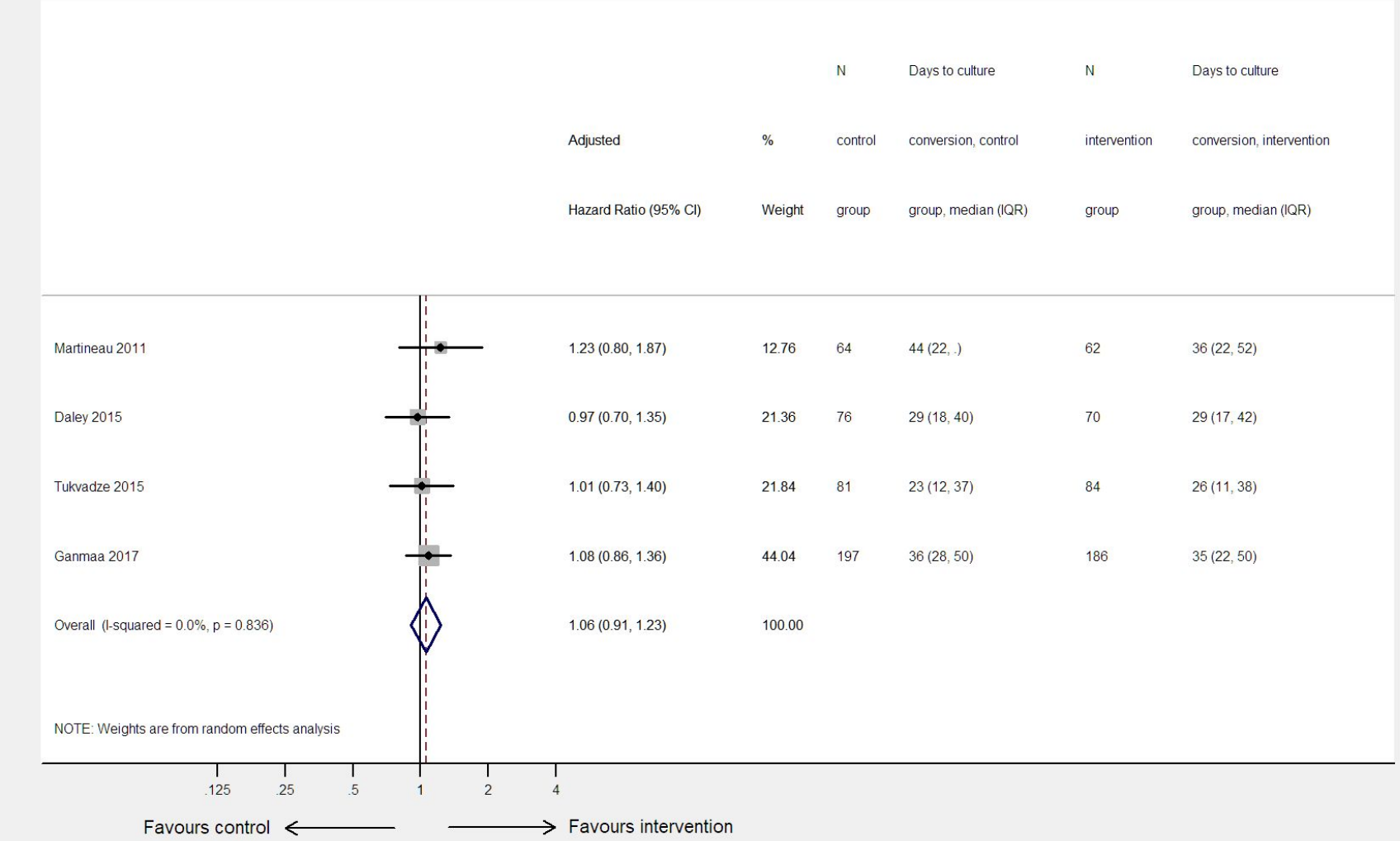


Figure 3: Time to sputum culture conversion by allocation: two-step individual participant data meta-analysis. Hazard ratios are adjusted for age and sex.



Adjunctive vitamin D in tuberculosis treatment: meta-analysis of individual participant data. On-line data supplement.

Search Strategies.

A. PubMed

Cochrane Highly Sensitive Search Strategy for identifying randomized controlled trials

#1. randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized [tiab] OR placebo [tiab] OR drug therapy [sh] OR randomly [tiab] OR trial [tiab] OR groups [tiab]

#2. animals [mh] NOT humans [mh]

#3. #1 NOT #2

Terms specific to vitamin D

#4. Vitamin D OR vitamin D2 OR vitamin D3 OR cholecalciferol OR ergocalciferol OR alphacalcidol OR alfacalcidol OR calcitriol OR paricalcitol OR doxerocalciferol

Term specific to tuberculosis

#5. Tuberculosis

Combination of terms to identify randomized controlled trials of vitamin D conducted in patients with tuberculosis

#3 AND #4 AND #5

B. EMBASE

Terms for identifying randomized controlled trials

#1 'randomized controlled trial'/exp OR 'single blind procedure'/exp OR 'double blind procedure'/exp OR 'crossover procedure'/exp
#2 random*:ab,ti OR placebo*:ab,ti OR crossover*:ab,ti OR 'cross over':ab,ti OR allocat*:ab,ti OR ((singl* OR doubl*) NEXT/1 blind*):ab,ti OR trial:ti
#3. #1 OR #2

Terms specific to vitamin D

#4. vitamin AND d OR vitamin AND d2 OR vitamin AND d3 OR cholecalciferol OR ergocalciferol OR alphacalcidol OR alfacalcidol OR calcitriol OR paricalcitol OR doxerocalciferol

Term specific to tuberculosis

#5. tuberculosis

Combination of terms to identify randomized controlled trials of vitamin D conducted in patients with tuberculosis

#3 AND #4 AND #5

C. Cochrane Central

Terms specific to vitamin D

#1. Vitamin D OR vitamin D2 OR vitamin D3 OR cholecalciferol OR ergocalciferol OR alphacalcidol OR alfacalcidol OR calcitriol OR paricalcitol OR doxerocalciferol

Term specific to tuberculosis

#2. Tuberculosis

Combination of terms to identify randomized controlled trials of vitamin D conducted in patients with tuberculosis

#1 AND #2

D. Web of Science

TS =(Vitamin D OR vitamin D2 OR vitamin D3 OR cholecalciferol OR ergocalciferol OR alphacalcidol OR alfacalcidol OR calcitriol OR paricalcitol OR doxerocalciferol) AND TS =(Tuberculosis) AND TS =(placebo* or random* or clinical trial* or double blind* or single blind* or rct)

Results

Appendix Table 1: Final treatment outcome by study and allocation[1]

	Ralph *et al*[1]		Wejse *et al*[2]	
	Placebo (n)	Vitamin D (n)	Placebo (n)	Vitamin D (n)
Cured	32	36	59	72
Completed	6	4	34	29
Failed	1	0	0	0
Defaulted	0	5	14	9
Died	0	2	9	12
Transferred	2	2	1	2
Total	**41**	**49**	**117**	**124**

1, data on final treatment outcome were available for 90 participants in the trial by Ralph et al and 241 participants in the trial by Wejse et al.

Appendix Table 2: Baseline characteristics by allocation: all analyzed participants (n=1,850)

	Placebo group (n=923)	Vitamin D group (n=927)
Mean baseline 25(OH)D concentration, nmol/L (SD)[1]	41.0 (35.7)	41.3 (33.6)
Baseline 25(OH)D concentration (%)[1]		
<25 nmol/L	359 (38.9)	329 (35.4)
≥25 nmol/L	478 (51.8)	494 (53.3)
Mean age, years (SD)	34.0 (13.8)	33.0 (13.0)
Age (%)		
<30 years	450 (48.8)	467 (50.4)
≥30 years	473 (51.2)	460 (49.6)
Sex (%)		
Male	611 (66.2)	589 (63.5)
Female	312 (33.8)	338 (36.5)
Mean weight, kg (SD)[2]	51.2 (11.3)	51.0 (10.9)
Multidrug resistance (%)[3]		
No	619 (67.1)	600 (64.7)
Yes	25 (2.7)	30 (3.2)
HIV status[4]		
Seronegative	507 (54.9)	492 (53.1)
Seropositive	41 (4.4)	68 (7.3)
% zones involved, baseline CXR (%)[5]		
<50%	362 (39.2)	359 (38.7)
≥50%	146 (15.8)	137 (14.8)
Cavitation, baseline CXR (%)[6]		
No	330 (35.8)	341 (36.8)
Yes	334 (36.2)	324 (35.0)
Vitamin D Dosing Regimen (%)		
Daily/weekly	223 (24.2)	229 (24.7)
Bolus/2-weekly	700 (75.8)	698 (75.3)
***Fok*I *VDR* genotype (%)**[7]		
FF	183 (19.8)	194 (20.9)
Ff	150 (16.3)	149 (16.1)
ff	36 (3.9)	20 (2.2)
***Taq*I *VDR* genotype (%)**[8]		
TT	258 (28.0)	245 (26.4)
Tt	140 (15.2)	140 (15.1)
tt	29 (3.1)	32 (3.5)

25(OH)D, 25-hydroxyvitamin D; CXR, chest radiograph; SD, standard deviation; HIV, human immunodeficiency virus; VDR, vitamin D receptor.

1. Data available for n=837 in the placebo group and n=823 in the vitamin D group; 2. Data available for n=906 in the placebo group and n=914 in the vitamin D group; 3. Data available for n=644 in the placebo group and n=630 in the vitamin D group; multidrug resistance defined as being resistant to both isoniazid and rifampicin, at least; 4. Data available for n=548 in the placebo group and n=560 in the vitamin D group; 5. Data available for n=408 in the placebo group and n=496 in the vitamin D group; 6. Data available for n=664 in the placebo group and n=665 in the vitamin D group; 7. Data available for n=369 in the placebo group and n=363 in the vitamin D group; 8. Data available for n=427 in the placebo group and n=417 in the vitamin D group.

Appendix Table 3: Baseline characteristics by allocation: participants with multidrug-resistant tuberculosis (n=55)[1]

		Placebo group (n=25)	Vitamin D group (n=30)
Mean age, years (SD)		30.7 (7.8)	31.8 (13.1)
Age (%)	<30 years	13 (52.0)	19 (63.3)
	≥30 years	12 (48.0)	11 (36.7)
Sex (%)	Male	15 (60.0)	16 (53.3)
Male	Female	10 (40.0)	14 (46.7)
Mean baseline 25(OH)D concentration, nmol/L (SD)[2]		21.6 (16.3)	26.7 (23.8)
Baseline 25(OH)D concentration (%)	<25 nmol/L	17 (68.0)	17 (56.7)
	≥25 nmol/L	7 (28.0)	12 (40.0)
	Not known	1 (4.0)	1 (3.3)
Mean weight, kg (SD)		54.1 (11.1)	55.5 (9.7)
HIV status (%)	Seronegative	11 (44.0)	13 (43.3)
	Seropositive	0 (0.0)	1 (3.3)
	Not known	14 (56.0)	16 (53.3)
Cavitation, baseline CXR (%)	No	13 (52.0)	21 (70.0)
	Yes	12 (48.0)	8 (26.7)
	Not known	0 (0.0)	1 (3.3)
Vitamin D dosing Regimen (%)	Daily/weekly	15 (60.0)	15 (50.0)
	Bolus/2-weekly	10 (40.0)	15 (50.0)
Other adjunctive therapy (%)	Phenylbutyrate	3 (12.0)	1 (3.3)
	L-arginine	1 (4.0)	0 (0.0)
	Nil	21 (84.0)	29 (96.7)
***FokI VDR* genotype (%)**[5]	FF	2 (8.0)	6 (20.0)
	Ff	5 (20.0)	6 (20.0)
	ff	2 (8.0)	1 (3.3)
	Not known	16 (64.0)	17 (56.7)
***TaqI VDR* genotype (%)**[6]	TT	9 (36.0)	13 (43.3)
	Tt	3 (12.0)	1 (3.3)
	tt	4 (16.0)	1 (3.3)
	Not known	9 (36.0)	15 (50.0)
Antimicrobial sensitivity	Resistant to isoniazid and rifampicin only	10 (40.0)	15 (50.0)
	Additionally resistant to at least one other anti-TB drug[3]	15 (60.0)	15 (50.0)

25(OH)D, 25-hydroxyvitamin D; CXR, chest radiograph; SD, standard deviation; HIV, human immunodeficiency virus; VDR, vitamin D receptor.

1. Multidrug-resistant tuberculosis defined as resistance of isolate to both isoniazid and rifampicin, at least.
2. Baseline 25(OH)D concentration missing for 1 participant in the placebo group and 1 participant in the vitamin D group

3. Isolates additionally resistant to ethambutol, streptomycin or both

Appendix Table 4. Risk of Bias Assessment

	Sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Wejse 2009 [2]	✓	✓	✓	✓	?[1]	✓	✓
Martineau 2011 [3]	✓	✓	✓	✓	✓	✓	✓
Salahuddin 2013 [4]	✓	✓	✓	✓	✓	✓	✓
Ralph 2013 [1]	✓	✓	✓	✓	?[1]	✓	✓
Mily 2015 [5]	✓	✓	✓	✓	?[1]	✓	✓
Daley 2015 [6]	✓	✓	✓	✓	?[1]	✓	✓
Tukvadze 2015 [7]	✓	✓	✓	✓	✓	✓	✓
Ganmaa 2017 [8]	✓	✓	✓	✓	✓	✓	✓

✓ = low risk of bias; ? = unclear risk of bias;
1, risk of bias due to incomplete outcome data assessed as 'unclear' due to relatively high rates of loss to follow-up (>20%)

Appendix Table 5: One-step individual participant data meta-analysis, safety outcomes

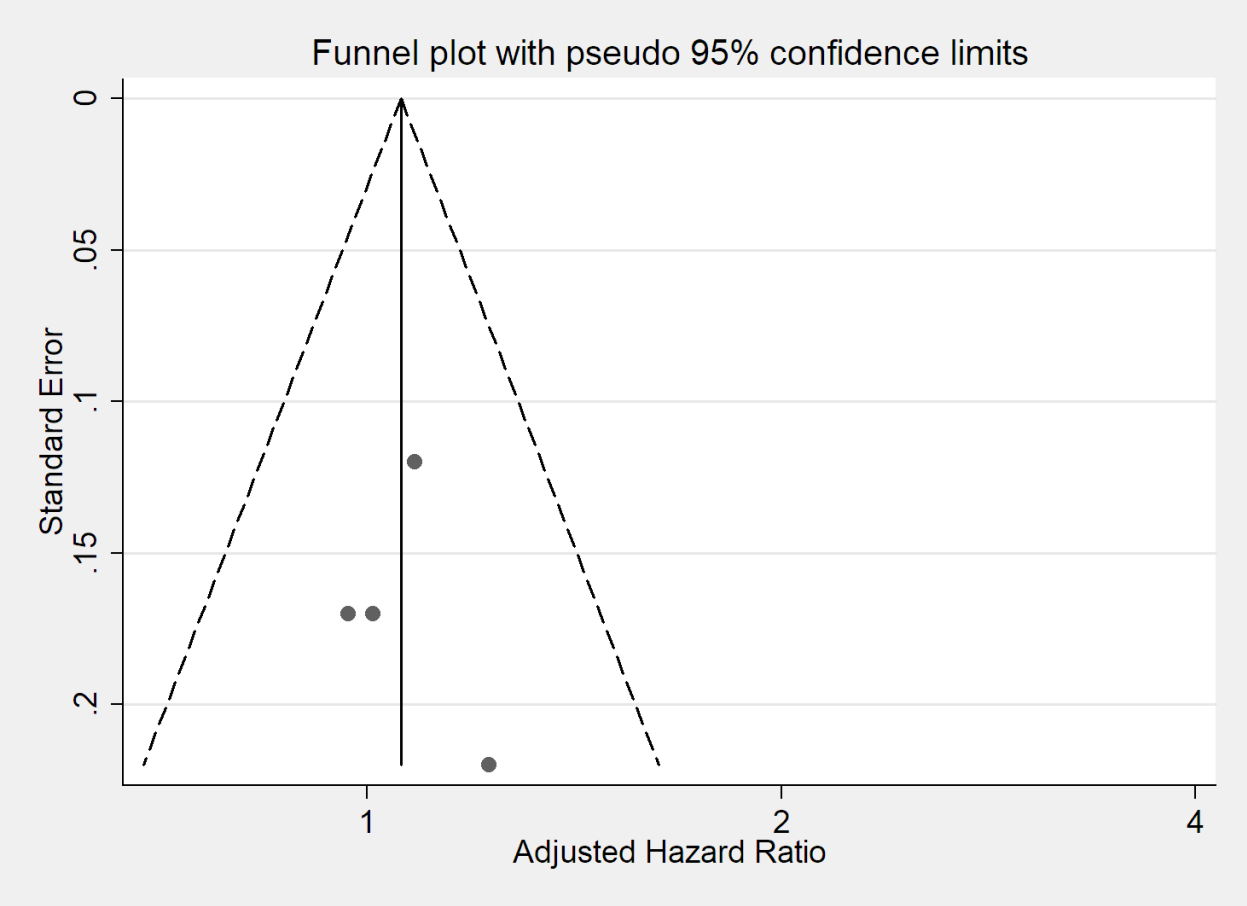
	N participants (trials)	Proportion with ≥1 event, placebo group (%)	Proportion with ≥1 event, vitamin D group (%)	Adjusted odds ratio (95% CI) [1]	P value
Hypercalcemia[2]	6	39/639 (6.1)	38/614 (6.2)	0.94 (0.55, 1.62)	0.83
Renal stones	8	0/923 (0.0)	0/927 (0.0)	--	--
Serious Adverse Events, any cause	8	32/923(3.5)	33/927 (3.6)	0.99 (0.59, 1.65)	0.96
Withdrawals/loss to follow-up	8	124/923 (13.4)	117/927 (12.6)	0.96 (0.73, 1.27)	0.79
Total Deaths	8	21/923 (2.3)	23/927 (2.5)	1.09 (0.59, 2.03)	0.79

1, adjusted for age, sex and clustering between trials
2, hypercalcemia defined as serum calcium concentration >2.65 mmol/L; serum calcium concentration was corrected for serum albumin concentration where this was also measured (4/6 studies). In all studies where serum calcium concentrations were measured, this was done routinely i.e. irrespective of symptoms

Appendix Table 6: One-step individual participant data meta-analysis, responder analysis

	N participants (N trials)	Median time to sputum culture conversion, days (IQR)	Adjusted hazard ratio (95% CI)	P value
Intervention, end-study 25(OH)D < 75 nmol/L	34 (3)	28 (28, 56)	Referent	
Intervention, end-study 25(OH)D ≥ 75 nmol/L	232 (4)	28 (28, 53)	0.95 (0.63, 1.41)	0.78

Appendix Figure 1: Funnel plot for individual patient data meta-analysis of time to sputum culture conversion.



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