Daily adjunctive therapy with vitamin D₃ and phenylbutyrate supports clinical recovery from pulmonary tuberculosis: a randomized controlled trial in Ethiopia

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Abstract. Bekele A, Gebresellassie N, Ashenafi S, Kassa E, Aseffa G, Amogne W, Getachew M, Aseffa A, Worku A, Raqib R, Agerberth B, Hammar U, Bergman P, Aderaye G, Andersson J, Brighenti S (Addis Ababa University, Addis Ababa, Ethiopia; Karolinska Institutet, Stockholm, Sweden; Armawer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia; International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh; Karolinska University Hospital Huddinge, Stockholm, Sweden). Daily adjunctive therapy with vitamin D₃ and phenylbutyrate supports clinical recovery from pulmonary tuberculosis: a randomized controlled trial in Ethiopia. J Intern Med 2018; 284: 292–306.

Objective. Immunotherapy using vitamin D (vitD₃) and phenylbutyrate (PBA) may support standard drug regimens used to treat infectious diseases. We investigated if vitD₃ + PBA enhanced clinical recovery from pulmonary tuberculosis (TB).

Methods. A randomized controlled trial was conducted in Addis Ababa, Ethiopia. Patients with smear-positive or smear-negative TB received daily oral supplementation with 5000 IU vitD₃ and 2 x 5000 mg PBA or placebo for 16 weeks, together with 6-month chemotherapy. Primary end-point: reduction of a clinical composite TB score at week 8 compared with baseline using modified intention-to-treat (mITT, n = 348) and per-protocol (n = 296) analyses. Secondary end-points: primary and modified TB scores (week 0, 4, 8, 16, 24), sputum conversion, radiological findings and plasma 25(OH)D₃ concentrations.

Results. Most subjects had low baseline plasma 25(OH)D₃ levels that increased gradually in the vitD₃ + PBA group compared with placebo (P = 0.0001) from week 0 to 16 (mean 34.7 vs. 127.4 nmol L⁻¹). In the adjusted mITT analysis, the primary TB score was significantly reduced in the intervention group at week 8 (difference -0.52, 95% CI -0.93, -0.10; P = 0.015) while the modified TB score was reduced at week 8 (difference -0.58, 95% CI -1.02, -0.14; P = 0.01) and 16 (difference -0.34, 95% CI -0.64, -0.03; P = 0.03). VitD₃ + PBA had no effect on longitudinal sputum-smear conversion (P = 0.98). Clinical adverse events were more common in the placebo group (24.3%) compared with the vitD₃ + PBA group (12.6%).

Conclusion. Daily supplementation with vitD₃ + PBA may ameliorate clinical TB symptoms and disease-specific complications, while the intervention had no effect on bacterial clearance in sputum.

Keywords: clinical trial, host defence, phenylbutyrate, tuberculosis, vitamin D.

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Trial registration. ClinicalTrials.gov NCT01698476
Introduction

New drug regimens for tuberculosis (TB) are necessary both to prevent TB disease and to treat ongoing active TB [1]. In contrast to single molecular targets, targeting multiple pathways in an attempt to treat chronic infections may reduce the risk of drug-resistance and clinical complications. Immunomodulatory compounds such as vitamin D3 (vitD3) and phenylbutyrate (PBA) are attractive therapeutic candidates with the ability to regulate various axes of the immune system [2]. In vitro, vitD3 can enhance macrophage-mediated killing of Mycobacterium tuberculosis (Mtb) by inducing the antimicrobial peptide LL-37 [3, 4] and autophagy [5]. LL-37 may also exert chemotactic functions to activate migration of immune cells to the site of infection [6]. Similarly, PBA, which is a histone deacetylase (HDAC) inhibitor, induces LL-37 in different cell types [7] and autophagy in macrophages [8] but also exhibits direct bacteriostatic effects on Mtb [9]. Accordingly, the combination of vitD3 and PBA has been shown to enhance intracellular Mtb-killing in vitro [8] and ex vivo [10, 11]. While both vitD3 and PBA are potent inducers of innate mucosal immunity, these compounds also possess important anti-inflammatory properties including inhibition of dendritic cell maturation, Th1/Th17 cell proliferation and cytokine production [2, 12]. Thus, hypothetically, vitD3 + PBA has the potential to reduce bacterial growth and simultaneously resolve pathological inflammation in the Mtb-infected lung.

To test if adjunct therapy with vitD3 + PBA could support clinical recovery in pulmonary TB, we performed a randomized controlled trial in Ethiopia. As large bolus doses of vitD3 generally fail to improve TB outcomes [13–15], we used daily dosing of vitD3 in combination with PBA for 16 weeks of chemotherapy. Bacteriological confirmation of pulmonary TB mostly involves detection of acid-fast bacilli (AFB) or Mtb-growth in sputum. However, about 20–50% of pulmonary TB patients are smear-negative [16], but qualify for initiation of standard chemotherapy based on a high clinical suspicion and radiographic findings according to the World Health Organization (WHO) criteria [1]. Thus, we designed a longitudinal study to exploit if vitD3 + PBA could improve clinical status in smear-positive and smear-negative TB patients by limiting bacterial load and pathological inflammation in the lung. The response to adjunct vitD3 + PBA therapy was evaluated using a numerical composite TB score, assessing the reduction in clinical symptoms during the initial 8-week intensive-phase treatment with standard drugs. This is a validated score composed of 11 clinical variables used to measure TB patients’ clinical status at repeated visits [17] and as an outcome measure in clinical trials [13, 18, 19]. Symptoms effectively improve during the initial 8-week intensive-phase standard treatment as a result from rapidly reduced bacterial loads in the lung and bacterial clearance from sputum in about 80% of TB patients [1, 20], and therefore this time-point was used to assess the primary outcome.

Materials and methods

For details on the Methods, please see the online Appendix S1.

Study design

A randomized, double-blinded, placebo-controlled trial was conducted at the Chest Unit, Department of Internal Medicine, Black Lion University Hospital in Addis Ababa, Ethiopia in collaboration with 11 health centres after ethical approval in Ethiopia and Sweden (Appendix S1). The study was registered at www.clinicaltrials.gov, NCT01698476, prior to inclusion of the first patient.

Patients

Inclusion criteria: HIV-negative patients >18 years, with newly diagnosed pulmonary TB (<5 days chemotherapy). Diagnoses were made from: (i) positive sputum-smear microscopy or Mtb-culture, and/or (ii) clinical symptoms and chest X-ray findings consistent with TB, that is clinical TB defined according to WHO criteria. Exclusion criteria: HIV infection, multidrug-resistant TB (MDR-TB) or extrapulmonary TB, anti-TB treatment in the past 2 years, hypercalcemia (serum calcium > 3.0 mmol L−1), pregnancy and breastfeeding, liver or renal diseases, malignancies or treatment with cardiac glycosides. All patients provided written and signed informed consent before enrolment.

Interventions

This was a two-arm intervention trial using daily adjunct therapy with vitD3 + PBA during the first 16 weeks of 6-month standard chemotherapy including a fixed-dose combination of isoniazid,
rifampicin, pyrazinamide and ethambutol for 8 weeks (intensive-phase treatment) and isoniazid and rifampicin for an additional 16 weeks. Patients were randomized to receive daily oral supplementation using the following dosing scheme [10, 21]: (i) 5000 IU vitD3 (five tablets once daily) and 500 mg PBA (one tablet twice daily), or (ii) vitD3 placebo and PBA placebo tablets. VitD3 tablets were used instead of oil, to control for variations in self-dosage of the oil preparation. Good manufacturing practice-produced vitD3 tablets were donated by Merck Serono (Darmstadt, Germany); PBA (Sodium Phenylbutyrate) and matching placebo were obtained from Scandinavian Formulas (Sellersville, PA, USA).

Randomization and masking

Subjects were randomized in a one-to-one allocation ratio using computer-generated randomization codes and block randomization with a block size of 10 (Karolinska Trial Alliance, Stockholm, Sweden), to ensure that in each block, five subjects were randomized to vitD3 + PBA and the other five subjects to placebo. Pharmacists at the Black Lion Hospital prepared the study medication and provided the randomization codes that assigned the patients to vitD3 + PBA or placebo treatment. Patients were recruited by senior consultants and a health officer, and they were all blinded to the randomization.

Outcome measures

The primary end-point was clinical recovery, assessed as the reduction/change of clinical symptoms at week 8 compared with week 0 (baseline). As it is not possible to record improvement of TB disease using a single symptom or laboratory result, we used a previously validated clinical TB score [13, 17]. This is a numerical composite TB score (2-point scale: symptom absent (0p) or present (1p), max 13p) that included self-reported clinical symptoms (cough, night sweats and chest pain), as well as different variables monitored by the study physician upon clinical examination [anaemia, hemoptysis, dyspnoea, tachycardia, positive findings at lung auscultation, fever, low body mass index (BMI) and low mid-upper arm circumference (MUAC)]. The primary TB score was also grouped into different severity classes as mild (SC-I: 0–5p), moderate (SC-II: 6–7) and severe (SC-III: ≥8p) disease [17].

Secondary end-points included longitudinal assessments of the primary and a modified TB score (week 0, 4, 8, 16 and 24), sputum-smear microscopy (week 0–4, and 8) and Mtb-culture (week 0 and 8) conversion, chest X-ray (week 0, 4, 8, 16 and 24), and levels of 25-hydroxyvitamin D3 (25(OH)D3) in plasma (week 0, 4, 8 and 16). The modified TB score (3-point scale: symptom absent (0p), improved (1p) or no change/worsened (2p), max 22p) was generated using a more spread grading scale of the primary TB score, aiming to detect and include small but important changes in clinical symptoms (Table S1).

Procedures

Sputum and blood samples were collected for the described laboratory analyses. Sputum-smear microscopy and sputum-culture, erythrocyte sedimentation rate, total and differential counts, CD4 T-cell counts (BD Biosciences, Franklin Lakes, NJ, USA) and blood chemistry analyses were conducted at the International Clinical Laboratory (ICL), which is a RANDX International Quality Assessment Scheme (RIQAS)-accredited and Centers for Disease Control and Prevention (CDC)-certified commercial laboratory in Addis Ababa, Ethiopia. Adverse events (AEs) included examinations of TB-specific clinical complications (week 4, 8, 16 and 24) and blood chemistry analysis (week 0, 4, 8 and 16) to measure liver and kidney function and calcium/phosphate homeostasis. QuantiFERON-TB Gold in-Tube (Cellectis; Statens Serum Institut, Denmark) was assessed in whole blood samples at the Armaver Hansen Research Institute (AHRI) in Addis Ababa, Ethiopia, according to the manufacturer’s instructions, for detection of Mtb-specific IFN-γ release in vitro. Levels of 25(OH)D3 in plasma samples were analysed at the Department of Clinical Chemistry, Karolinska University Hospital in Stockholm, Sweden using a chemiluminescence immunoassay on a LIAISON-instrument (DiaSorin Inc., Stillwater, MN, USA), detectable range 7.5–175 nmol L⁻¹, CV 2–5%. Plasma 25(OH)D3 concentrations were used to determine vitD3 status and to monitor treatment adherence.

Statistical analysis

The sample size calculation was based on a previous study demonstrating that standard TB care will reduce the primary clinical TB score from 6.5 to 3.2 during the initial 8-week intensive-phase treatment [17]. To reduce the TB score, an
additional 25% above the effect of standard chemotherapy at 8 weeks (calculating with a mean TB score of 3.2 in the placebo group and a standard deviation of 2.3 in both intervention groups), a sample size of 131 patients/group was required (80% power, \(P < 0.05\), two-sided test). The power calculations included 8-week data alone. But as described in the original study protocol, analyses of the primary end-point were based on a comparison of the change in the TB score between baseline (week 0) and week 8 in the two study groups, which likely increased the power of the analyses. Assuming a dropout rate of approximately 15%, the sample size was increased to 300 patients. To compensate for the proportion of patients with sputum-negative clinical TB to patients with sputum-smear-positive TB, the sample size was increased another 20%, resulting in 360 patients.

Results were analysed following the intention-to-treat (ITT)-concept, using multiple imputation by chained equations to impute outcomes for persons lost to follow-up. We applied modified ITT (mITT) analysis, which is commonly used in antimicrobial/anti-infective trials, when test results obtained after randomization show that some patients were misdiagnosed and/or ineligible. mITT allows for these randomized subjects to be excluded from the analysis in a justified way. Per-protocol analyses included all subjects who completed the study treatment. Primary and secondary analyses were conducted using linear regression, ordinal logistic regression (AFB-grading and radiology) and logistic regression (Mtbculture conversion). Both crude and adjusted analyses were made. The covariates adjusted for were age, gender, smear positivity and baseline value of the outcome. Those variables were selected \(a \text{ priori}\) to increase the precision of our estimates, as we believed them to be associated with the outcome [22]. Time to sputum-microscopy conversion was shown using a Kaplan–Meier-plot and a log rank test. A \(P\) value <0.05 was considered significant. Analyses were conducted using IBM SPSS Statistics 20.0 and Stata 13 (StataCorp, College Station, TX, USA).

Results

Enrolment

We screened 894 patients for eligibility from January 2013 to May 2015 as described in the trial profile (Fig. 1). Most patients ineligible for randomization were HIV-infected (\(n = 418\)). After randomization of 390 patients, laboratory testing confirmed that 42 enrolled patients did not fulfil the predefined exclusion criteria [other pulmonary diseases (\(n = 8\)) and MDR-TB (\(n = 16\))]. The remaining 348 subjects constituted the mITT cohort, allocated to \(\text{vitD}_3 + \text{PBA}\) (\(n = 175\)) or placebo (\(n = 173\)) treatment. A total of 52 patients discontinued the intervention or were lost to follow-up (dropout rate = 14.9%). Thus, 296 patients completed the treatment per-protocol, allocated to \(\text{vitD}_3 + \text{PBA}\) (\(n = 150\)) or placebo (\(n = 146\)) treatment.

Baseline characteristics

Baseline data are presented in Table 1. About 81% of all patients had a positive sputum-smear and/or Mtbculture result, out of which 10–12% were discordant samples (Table 1). Additional 19% of the patients had a clinical TB diagnosis, of whom, 94% had a positive QuantiFERON result (Appendix S1). Around 50% of the patients had a BMI below 18 \(\text{kg m}^{-2}\) while 54% had a MUAC below 22 cm, indicating underweight (Table 2). The primary TB score had a mean of 5-6p, grouping 49.5% of the patients into severity class II-III. Plasma 25(OH)D3 concentrations were low, around 35 nmol L\(^{-1}\). As an international reference for 25(OH)D3 concentrations in blood, we followed the Endocrine Society’s Clinical Practice Guideline defining \(\text{vitD}_3\) deficiency as 25(OH) D3 levels below 50 nmol L\(^{-1}\) [23]. Accordingly, most TB patients were \(\text{vitD}_3\)-deficient (80.6%) or insufficient (15.0%) at baseline. From Table 1, it is also evident that the distribution of gender was slightly skewed. Gender was one of the covariates adjusted for in the primary and secondary analyses.

Primary end-point: clinical TB score

In the TB score, cough, night sweats, chest pain, tachycardia, low BMI and low MUAC were the most common clinical symptoms, while conjunctiva palor, haemoptysis and fever were less frequent (Table 2). Longitudinal assessments of the primary TB score are illustrated in Fig. 2a and Figure S1, and the differences and 95% CI are shown in Table 3. In the adjusted mITT analysis, the primary TB score was significantly reduced at week 8 (\(P = 0.015\)) and the modified TB score was significantly reduced at weeks 8 (\(P = 0.01\)) and 16.
(P = 0.03) in the vitD3 + PBA group compared with placebo. Similarly, in the adjusted per-protocol analysis, we observed a significant reduction in both the primary (P = 0.022) and the modified (P = 0.016) TB score at week 8. Overall, the odds ratios of individual clinical symptoms predominantly favoured the treatment group (Fig. 2b).

Secondary end-points: sputum-conversion analyses and 25(OH)D3 levels in plasma

Sputum-conversion rates and AFB-grading are illustrated in Fig. 3 and the odds ratio and 95% CI are shown in Table 4. Longitudinal analysis showed no significant effect of vitD3 + PBA treatment on the time to sputum-microscopy conversion in smear-positive patients (P = 0.98) (Fig. 3a). However, AFB-grading demonstrated a significant reduction in smear-positive TB in the intervention group at week 4 using both mITT (P = 0.017 and P = 0.037) and per-protocol analyses (P = 0.024 and P = 0.038), although this difference was no longer detected at week 8 (Fig. 3b and Table 3). Neither, could we detect enhanced Mtb-culture conversion (Fig. 3c) or radiological improvement (Figure S2).
### Table 1 Baseline characteristics

| Variables                              | Placebo (n = 173) | vitD₃ + PBA (n = 175) |
|----------------------------------------|-------------------|-----------------------|
| (mITT, n = 348)                        |                   |                       |
| Gender (M/F) (no/%)                    | 110 (64)/63 (36)  | 91 (52)/84 (48)       |
| Age (mean)                             | 30.63             | 30.31                 |
| Sputum-smear status (no/%)             |                   |                       |
| pos                                    | 120 (69.4)        | 116 (66.2)            |
| neg                                    | 53 (30.6)         | 59 (33.7)             |
| Sputum-culture status (no/%)           |                   |                       |
| pos                                    | 124 (71.7)        | 119 (68.0)            |
| neg                                    | 25 (14.4)         | 30 (17.1)             |
| NDA                                    | 25 (14.4)         | 26 (14.9)             |
| Clinical TB (no/%)                     | 31 (17.9)         | 35 (20.0)             |
| Pos QuantiFERON (no/%)                 | 30 (96.8)         | 32 (91.4)             |
| Duration of cough (weeks)              | 4 (3–8)           | 4 (3–8)               |
| History of contact (no/%)              | 35 (20.2)         | 45 (25.7)             |
| History of TB treatment (no/%)         | 17 (9.8)          | 8 (4.6)               |
| History of smoking (no/%)              | 23 (13.3)         | 33 (18.9)             |
| BCG vaccination (no/%)                 | 50 (28.9)         | 52 (29.7)             |
| Weight loss (no/%)                     | 127 (73.4)        | 111 (63.4)            |
| Weight loss (kg)                       | 5 (3–8)           | 5 (3–10)              |
| BMI (median)                           | 18 (17–19)        | 18 (17–20)            |
| MUAC (median)                          | 22 (20–23)        | 22 (21–24)            |
| Pulse rate/min (median)                | 87 (80–94)        | 83 (78–93)            |
| Respiratory rate/min (median)          | 20 (18–24)        | 20 (18–24)            |
| WBC (median)                           | 7 (6–9)           | 8 (5–10)              |
| ESR (median)                           | 42 (30–52)        | 46 (31–55)            |
| Haemoglobin (median)                   | 12 (12–15)        | 13 (12–14)            |
| Calcium (median)                       | 9 (8–9)           | 9 (8–10)              |
| Albumin (median)                       | 4 (3–4)           | 4 (3–4)               |
| TB score (median/IQR)                  | 6 (4–7)           | 5 (4–7)               |
| Primary TB score                       | 9 (7–12)          | 9 (6–12)              |
| SC-I: 0–5                              | 83 (48.0)         | 93 (53.1)             |

### Table 1 (Continued)

| Variables                              | Placebo (n = 173) | vitD₃ + PBA (n = 175) |
|----------------------------------------|-------------------|-----------------------|
| (mITT, n = 348)                        |                   |                       |
| SC-II: 6–7                             | 56 (32.4)         | 44 (25.1)             |
| SC-III: ≥8                             | 34 (19.6)         | 38 (21.7)             |
| 25(OH)D₃ nmol L⁻¹ (mean)               | 35.47             | 34.72                 |
| Deficiency <50 nmol L⁻¹ (no/%)         | 135 (78.0)        | 144 (83.2)            |
| Insufficiency                          | 31 (17.9)         | 21 (12.1)             |
| 50–75 nmol L⁻¹                          | 0 7 (4.0)         | 8 (4.6)               |

BCG, Bacillus Calmette Guerin; BMI, body mass index; ESR, erythrocyte sedimentation rate; IQR, interquartile range; mITT, modified intention-to-treat; MUAC, mid-upper-arm-circumference; NDA, no data available; SC, severity class; WBC, white blood cell; 25(OH)D₃, 25-hydroxyvitamin D.

aData are n (%), mean or median (IQR).

bSputum-microscopy and sputum-culture positivity were not always overlapping, but around 10–12% of the samples were discordant.

bThree patients had a negative QuantiFERON; vitD₃ + PBA (n = 2) and placebo (n = 1), and one patient had no QuantiFERON test taken, vitD₃ + PBA (n = 1).

dTreatment with anti-TB drugs >2 years before study enrolment.

Most subjects had low plasma 25(OH)D₃ levels at baseline that increased significantly (P < 0.0001) in the vitD₃ + PBA group compared with placebo at week 4 (mean 38.6 vs. 91.5 nmol L⁻¹), week 8 (mean 38.4 vs. 109.4 nmol L⁻¹), and week 16 (mean 40.1 vs. 127.4 nmol L⁻¹) (Fig. 4), which indicated good adherence. Baseline levels of 25(OH)D₃ also increased significantly (P = 0.0014) in the placebo group at week 16 (mean 35.5 vs. 39.9 nmol L⁻¹), although this increase was modest compared with the increase in the vitD₃ + PBA group at week 16 (4.4 nmol L⁻¹ vs. 92.7 nmol L⁻¹). Thus, vitD₃ deficiency in the vitD₃ + PBA group was rapidly corrected and most patients reached optimal 25(OH)D₃ levels >75 nmol L⁻¹ within 4 weeks of vitD₃ + PBA supplementation. Interestingly, patients who raised their baseline 25(OH)D₃ levels significantly more likely to have reduced AFB in sputum compared with patients who maintained low 25(OH)D₃ levels (P = 0.005 and P = 0.008).
Thus, the odds of an AFB-positive sputum sample were reduced with 2% per unit increase in 25(OH)D₃ concentration.

**Table 2** Baseline data in clinical TB scores

| Variables                        | Placebo (mITT, n = 348) | vitD₃ + PBA (n = 348) |
|----------------------------------|--------------------------|-----------------------|
| Cough (0/1)                      | 8/165                    | 3/172                 |
| Night sweats (0/1)               | 30/143                   | 29/146                |
| Chest pain (0/1)                 | 61/112                   | 63/112                |
| Conjunctiva pallor (0/1)         | 157/16                   | 146/28                |
| Anaemia (Hb, mg dL⁻¹) (0/1)      | 119/54                   | 116/59                |
| Haemoptysis (0/1)                | 145/28                   | 149/26                |
| Dyspnoea (0/1)                   | 102/71                   | 102/73                |
| Tachycardia (0/1)                | 81/92                    | 78/97                 |
| Lung auscultations (0/1)         | 116/57                   | 106/69                |
| Fever (0/1)                      | 166/7                    | 169/6                 |
| BMI <18 (0/1)                    | 77/96                    | 95/80                 |
| MUAC <220 cm (0/1)              | 71/102                   | 90/85                 |

BMI, body mass index; Hb, haemoglobin; mITT, modified intention-to-treat; MUAC, mid-upper-arm-circumference.

*A Clinical symptoms in the primary TB score are reported as absent (0) or present (1). Data are numbers.

Subgroup analyses: Clinical TB score in patients with vitD₃ deficiency and moderate-to-severe TB

A subgroup analysis showed that vitD₃ + PBA treatment was most beneficial in vitD₃-deficient patients with moderate-to-severe disease (TB score>5 i.e., severity class II-III) (P for interaction = 0.016) (Table S2). The primary and modified TB scores in this group are illustrated in Fig. 5, and the differences and 95% CI are shown in Table 3. Per-protocol analyses revealed a significant reduction in the primary TB score at week 8 (P = 0.005), while the modified TB score was significantly reduced at weeks 8 (P = 0.004 and P = 0.003) and 16 (P = 0.036) in the vitD₃ + PBA group. Furthermore, vitamin D responders, that is, TB patients with 25(OH)D₃ levels ≤50 nmol L⁻¹ at baseline and >75 nmol L⁻¹ at week 16 revealed a significant decrease in both primary and modified TB scores at week 8 (P = 0.034 and P = 0.014) and week 16 (P = 0.021 and P = 0.023) (Table S2).

**Fig. 2** Primary efficacy analyses. (a) The primary clinical TB score was assessed at baseline and at weeks 4, 8, 16 and 24 after initiation of anti-TB chemotherapy. Adjunct vitD₃ + PBA treatment was provided during the first 16 weeks of standard care. The efficacy analysis included comparison of the vitD₃ + PBA and placebo treatment between week 0 and week 8. Crude data from the mITT cohort are presented as the mean and 95% CI. The black line (circles) represents placebo while the grey line (triangles) represents vitD₃ + PBA treatment. The horizontal bar indicate the estimated difference (given a linear reduction of the TB score) in weeks that it would take to reduce the primary TB score in the placebo group to a level comparable to the TB score in the vitD₃ + PBA group assessed at the end of adjunct treatment at week 16. (b) Forrest plot showing the odds ratio of the individual diseases symptoms included in the primary efficacy analysis. The estimate and 95% CI at week 8 are shown. PBA, phenylbutyrate; mITT, modified intention-to-treat.

Adverse events

The major clinical AEs observed at follow-up were reported as TB-specific clinical complications
listed in Table 5. The most common manifestations in the treatment group were chest pain and anaemia while the placebo group patients commonly experienced chest pain, dyspnoea and dyspepsia. Significantly fewer clinical complications were reported in the vitD3 + PBA group compared with placebo (22 vs. 42; \( P = 0.006 \)). No clinically relevant changes in blood chemistry (calcium, phosphate, albumin or creatine) related to the intervention were observed (Tables S3 and S4).

**Discussion**

In this trial, we tested if daily adjunct therapy with vitD3 + PBA could improve clinical symptoms in smear-positive as well as smear-negative patients with pulmonary TB. Supplemented patients had an enhanced clinical recovery assessed as a reduction in clinical TB score during the first 8-weeks of intensive-phase treatment. The intervention did not influence time to sputum-microscopy conversion, although the odds of an AFB-positive result were significantly lower in the vitD3 + PBA group at week 4. The intervention was particularly effective in patients with low 25(OH)D3 levels and an elevated TB score at enrolment, suggesting that disease amelioration was more efficient in vitD3-deficient patients with more pronounced clinical symptoms. Moreover, it was safe to administer vitD3 + PBA daily for 16 weeks and clinical AEs were more common in the placebo group. We conclude that adjunct therapy with vitD3 + PBA may contribute to reduced disease severity and reduced clinical complications in patients with pulmonary TB, while the treatment had less effects on bacterial clearance \( \text{in vivo} \).

Our study has several limitations. A randomized study does not exclude the possibility of chance imbalances at baseline. In our study, this imbalance was observed in the somewhat skewed distribution of gender in the placebo compared with the

### Table 3  Clinical TB score in vitD3 + PBA versus placebo

| End-point      | Week | n  | Crude Difference 95% CI | P value | Adjusted\(^a\) Difference 95% CI | P value |
|----------------|------|----|-------------------------|---------|----------------------------------|---------|
| **All patients (mITT)** |      |    |                         |         |                                  |         |
| Primary TB score | 4    | 348| -0.07 -0.49 to 0.35     | 0.741   | -0.16 -0.54 to 0.23              | 0.425   |
|                 | 8    | 348| -0.42 -0.90 to 0.06     | 0.089   | -0.52 -0.93 to -0.10             | 0.015   |
|                 | 16   | 348| -0.17 -0.65 to 0.31     | 0.483   | -0.32 -0.65 to 0.00              | 0.051   |
| Modified TB score | 4    | 348| -0.13 -0.80 to 0.54     | 0.700   | -0.24 -0.77 to 0.29              | 0.375   |
|                 | 8    | 348| -0.48 -1.15 to 0.20     | 0.165   | -0.58 -1.02 to -0.14             | 0.010   |
|                 | 16   | 348| -0.19 -0.88 to 0.49     | 0.580   | -0.34 -0.64 to -0.03             | 0.030   |
| **Patients (per-protocol)** |      |    |                         |         |                                  |         |
| Primary TB score | 4    | 320| -0.10 -0.54 to 0.33     | 0.635   | -0.17 -0.55 to 0.25              | 0.454   |
|                 | 8    | 309| -0.42 -0.90 to 0.06     | 0.087   | -0.47 -0.86 to -0.07             | 0.022   |
|                 | 16   | 302| -0.22 -0.71 to 0.28     | 0.386   | -0.30 -0.61 to 0.01              | 0.055   |
| Modified TB score | 4    | 320| -0.21 -0.86 to 0.45     | 0.533   | -0.28 -0.82 to 0.26              | 0.315   |
|                 | 8    | 309| -0.53 -1.23 to 0.17     | 0.134   | -0.62 -1.11 to -0.11             | 0.016   |
|                 | 16   | 302| -0.27 -1.02 to 0.47     | 0.474   | -0.40 -0.81 to 0.02              | 0.060   |
| **Patients with 25(OH)D3 ≤50 nmol L\(^{-1}\) + TB score >5 (per-protocol)** |      |    |                         |         |                                  |         |
| Primary TB score | 4    | 125| -0.38 -1.10 to 0.34     | 0.296   | -0.46 -1.18 to 0.26              | 0.209   |
|                 | 8    | 120| -1.12 -1.90 to -0.34    | 0.005   | -1.11 -1.89 to -0.34             | 0.005   |
|                 | 16   | 118| -0.62 -1.29 to 0.05     | 0.070   | -0.61 -1.21 to 0.00              | 0.051   |
| Modified TB score | 4    | 125| -0.84 -1.74 to 0.06     | 0.066   | -0.89 -1.79 to 0.01              | 0.053   |
|                 | 8    | 120| -1.43 -2.39 to -0.47    | 0.004   | -1.37 -2.28 to -0.47             | 0.003   |
|                 | 16   | 118| -0.91 -1.83 to 0.01     | 0.052   | -0.83 -1.61 to -0.06             | 0.036   |

CI, confidence interval; mITT, modified intention-to-treat; 25(OH)D3, 25-hydroxyvitamin D.

\(^a\)Data are adjusted for gender, age, and TB score and sputum-smear positivity at baseline.
vitD₃ + PBA group. However, the adjusted analysis corrected for this imbalance. Furthermore, designing a two-arm intervention trial enabled an increased sample size per group, but prevented assessment of the individual effects of vitD₃ or PBA. Although the synergistic or additive effects of

Fig. 3 Sputum-smear conversion analyses. (a) Longitudinal analysis of time to sputum-smear conversion after initiation of anti-TB chemotherapy in patients who were sputum-microscopy positive at enrolment. Crude data are presented in a Kaplan-Meier curve. The black line represents placebo while the grey line represents vitD₃ + PBA treatment. The hazard ratio (HR) and 95% CI are shown. (b) AFB-grading among sputum-smear-positive TB patients at baseline compared to week 4 and 8 after initiation of anti-TB chemotherapy. AFB-positivity (+) was graded using microscopy as no AFB (negative), scanty (0–1), + 1, + 2, or + 3 AFB. Data are shown in a bar graph with a grey scale from 0 to 3+ AFB. The numbers and proportion of AFB + TB patients in the placebo versus vitD₃ + PBA group at week 4 and 8 are also indicated in the graph. Patients with a negative sputum-smear result at baseline were excluded from the conversion analysis. (c) Sputum-culture conversion among both Mtb-culture-positive and -negative TB patients at baseline compared to week 8 after initiation of anti-TB chemotherapy. Bar graph showing negative Mtb-culture (black) versus positive Mtb-culture (grey). PBA, phenylbutyrate.
these compounds have been well-described *in vitro* [7–9] and *ex vivo* [10, 11], additional clinical studies will contribute to an increased understanding of vitD3 + PBA treatment effects *in vivo*.

Furthermore, we used a semisoft end-point as primary outcome, a TB score that is a rapid, low-cost method for clinical monitoring of TB in resource-poor settings [17]. This validated score has been successfully used to follow prognosis and treatment outcome especially in smear-negative TB patients [13, 18, 19]. The score correlated with grade of smear positivity [17], and an elevated TB score at week 8 (i.e. SC-III), was associated to higher mortality and poor prognosis [19]. It is possible that smear-negative TB patients have a milder form of disease, including lower bacterial loads and less severe symptoms. Nevertheless, many of these patients start chemotherapy in line with WHO guidelines. Only including sputum-positive TB patients, representing 40–70% of all cases, may generate a selection bias that is not representative of standard clinical care. Thus, we maintain that a composite clinical score has advantages in measuring TB outcomes, particularly in routine clinical practice where sputum results are frequently negative.

It is difficult to show an effect of vitD3 + PBA on top of the highly effective standard chemotherapy. In this study, an additional 25% reduction in the TB score in the intervention group compared with the placebo group might be a meaningful effect size for clinical practice.

### Table 4  Sputum-smear conversion in vitD3 + PBA versus placebo

| End-point                                      | Week | n     | Crude OR | 95% CI  | P value | Adjusted OR | 95% CI  | P value |
|-----------------------------------------------|------|-------|----------|---------|---------|-------------|---------|---------|
| Smear-positive patients (mITT)                |      |       |          |         |         |             |         |         |
| Sputum-positivity                             | 4    | 199   | 0.45     | 0.23–0.87 | 0.017   | 0.49        | 0.25–0.94 | 0.037   |
|                                                | 8    | 199   | 1.07     | 0.43–2.65 | 0.879   | 1.05        | 0.42–2.63 | 0.904   |
| Smear-positive patients (per-protocol)        |      |       |          |         |         |             |         |         |
| Sputum-positivity                             | 4    | 173   | 0.46     | 0.23–0.90 | 0.024   | 0.48        | 0.24–0.95 | 0.038   |
|                                                | 8    | 174   | 1.29     | 0.48–3.42 | 0.616   | 1.29        | 0.48–3.47 | 0.584   |
| Smear-positive patients with elevated 25(OH)D3 levels at week 4 (per-protocol) |      |       |          |         |         |             |         |         |
| Sputum-positivity                             | 4    | 170   | 0.98     | 0.97–1.00 | 0.005   | 0.99        | 0.97–1.00 | 0.008   |

CI, confidence interval; mITT, modified intention-to-treat; OR, odds ratio.

aData are adjusted for gender, age and sputum-smear positivity at baseline.

**Fig. 4** VitD3 analysis. Plasma levels of 25(OH)D3 in the placebo compared with the vitD3 + PBA group at baseline and at weeks 4, 8, and 16 after initiation of anti-TB chemotherapy. Data are shown in a scatter dot plot with red symbols for placebo and grey symbols for vitD3 + PBA treatment. The solid line indicates the median, and the dashed lines mark the thresholds for vitD3 deficiency and insufficiency. PBA, phenylbutyrate.
standard drugs at 8 weeks was considered a significant effect. This change in TB score has previously been used to define clinical improvement [17]. The intervention had a significant effect on the composite TB scores, but not on any given symptom alone. The modified TB score was significantly reduced at both weeks 8 and 16 compared with week 8 for the primary TB score, also showing lower P values. This indicated that using a more nuanced grading scale (3-point instead of 2-point scale) of the validated primary TB score may increase the likelihood to detect changes in clinical symptoms among the study subjects. About 3–4 weeks extra time was required to reduce the primary TB score in the placebo group to a level comparable with the vitD3 + PBA group at the end of adjunctive therapy at week 16. At the end of standard chemotherapy at week 24, most patients had a TB score below 1, which suggested that the majority of clinical symptoms had disappeared due to the successful effects of lengthy 6-month standard care. Importantly, the reduction in both TB scores was more powerful in the subgroup analyses including one-third of the patients with vitD3 deficiency and more advanced TB disease, which strengthen the results of the primary analysis. Altogether, these data support the clinical relevance of our findings, although continued investigations will need to validate their applicability. Importantly, for a common infectious disease such as TB, even a small-to-moderate clinical effect on

### Table 5: Adverse events

| Manifestation                  | Placebo (n = 173) | vitD3 + PBA (n = 175) |
|-------------------------------|------------------|-----------------------|
| Chest pain                    | 8 (4.6)          | 6 (3.4)               |
| Dyspnoea                      | 9 (5.2)          | 2 (1.1)               |
| Anaemia                       | 3 (1.7)          | 5 (2.8)               |
| Numbness                      | 2 (1.2)          | 2 (1.1)               |
| Dyspepsia                     | 4 (2.3)          | 0                     |
| Night sweats                  | 1 (0.6)          | 3 (1.7)               |
| Haemoptysis                   | 3 (1.7)          | 0                     |
| Flank pain                    | 3 (1.7)          | 1 (0.6)               |
| Pneumonia                     | 2 (1.2)          | 0                     |
| Arthralgia                    | 1 (0.6)          | 1 (0.6)               |
| Exacerbated asthma            | 1 (0.6)          | 0                     |
| Oral rash                     | 1 (0.6)          | 0                     |
| Skin rash                     | 2 (1.2)          | 1 (0.6)               |
| Diarrhoea                     | 1 (0.6)          | 0                     |
| Ear discharge                 | 1 (0.6)          | 0                     |
| Breast abscess                | 0                | 1 (0.6)               |
| Total AEs^a                   | 42 (24.3)        | 22 (12.6)             |

AE, adverse event.  
^aAll AEs were grade 1 or mild, except for the oral rash that was classified as a grade 2 AE. All AEs were experienced by different individuals.
top of already existing standard treatment, may have significant positive effects on treatment outcome [24].

This study failed to show significant effects of vitD3 + PBA treatment on sputum conversion rates. The sensitivity and specificity of sputum-smear microscopy is limited, although this is the most common method for TB diagnosis and to follow treatment outcome [25]. Microscopy targets the most infectious cases with a threshold for Mtb detection of <10 000 bacilli mL⁻¹ of sputum, and therefore fails to diagnose clinical TB in many smear-negative patients [25]. Sputum-culture is more sensitive, but time-consuming and prevents grading of the bacterial load. Possibly, the standard anti-TB drugs are so effective to reduce bacterial growth that the potential antimycobacterial effects of vitD3 + PBA will be masked. Consequently, TB trial results could be misinterpreted if the primary effect of vitD3 + PBA is to modulate inflammatory responses or in other ways affect physiological processes that will improve clinical but not bacteriological outcomes [12, 26]. A recently described role for parent vitD3, the 25(OH)D₃ proform and the active 1,25(OH)₂D₃ metabolite, is to stabilize the endothelium, which is typically activated and destabilized during inflammation [27]. Interestingly, such vascular stabilization occurs independently from the antimicrobial effector functions triggered via intracellular vitD3 receptor signalling. Such effects may be better assessed using clinical improvement, resolution of inflammation and prevention of relapse.

This study also has several strengths. The majority of TB patients had a vitD3 deficiency at baseline that was rapidly corrected upon vitD3 + PBA treatment. Compelling evidence suggests that a low vitD3 status may enhance susceptibility to active TB [28, 29]. Importantly, basal 25(OH)D₃ levels can vary substantially between different populations and therefore TB patients may respond differently to vitD3 supplementation. TB patients in Tanzania [30], India [31] and Guinea-Bissau [13] had higher 25(OH)D₃ levels (ranges: 62–91 nmol L⁻¹), while patients in South Africa [28], Bangladesh [11], Pakistan [18] and the UK [14] were mostly vitD3-deficient (ranges: 20–34 nmol L⁻¹). Therefore, screening for vitD3 deficiency before start of standard treatment may increase the likelihood of successful adjunctive therapy with vitD3 and/or PBA.

Another strength was that daily doses of vitD₃ were administered together with PBA instead of using a bolus regimen. Due to the short half-life of parent vitD₃ (12–24 h), even large bolus doses are rapidly cleared from the circulation. Moreover, the cellular availability of vitD₃ and its proform is very different since 25(OH)D₃ is tightly bound to the vitD₃-binding protein, reducing cellular entry and activation compared with vitD₃ [32]. While daily dosing will sustain stable and physiological concentrations of circulating vitD₃, high-dose, long-interval dosing will result in large fluctuations in circulating vitD₃ concentrations [32]. The unfavourable consequences of such pharmacological dosing are underappreciated, as this will severely reduce a continuous supply of bioavailable intact vitD₃ as the major source for cellular uptake and conversion to the active metabolite that can maintain optimal functions of vitD₃-induced systems.

Until 2017, 11 randomized trials have been published investigating the therapeutic potential of adjunctive vitD₃ treatment in TB [11, 13–15, 18, 31, 33–37], but consensus on the potential beneficial effects is still lacking. Most trials were too small to demonstrate statistical power, the dosage regimen of vitD₃ was highly variable, as were baseline concentrations of 25(OH)D₃. The primary end-point was mainly time to sputum conversion, while treatment efficacy including smear-negative patients has rarely been reported. Importantly, most studies used bolus doses of vitD₃, which have consistently failed to support clinical and microbiological efficacy in TB [13–15, 31, 36, 37]. VitD₃ given at an early stage of chemotherapy [0, 14, 28, and 42 days] resulted in enhanced sputum conversion only in patients with the Taq Ⅰ tt genotype of the VDR [14], while vitD₃ provided at later timepoints (0, 5 and 8 months) failed to increase 25(OH)D₃ levels and accordingly had no effect compared to placebo [13]. Two doses of 200 000 IU vitD₃ (0 and 4 weeks) showed significant effects on weight gain, BMI and pulmonary involvement, but had no overall effect on the clinical TB score or smear conversion [18]. However, patients with 25(OH)D₃<30 nmol L⁻¹ at enrolment revealed significantly lower TB scores and a clear trend towards enhanced bacterial sputum clearance [18]. Similarly, daily vitD₃ + PBA treatment reduced both primary and modified TB scores more robustly in vitD₃-deficient patients with moderate-to-severe TB disease. Likewise, vitD₃ supplementation did not affect the time to first exacerbation in patients with COPD, but subgroup analysis revealed
significant effects in vitD3-deficient patients [38, 39]. Consistently, a recent meta-analysis provided evidence that daily-weekly administration of vitD3 reduced the risk of acute respiratory tract infections, particularly among individuals with low vitD3 levels [40]. Altogether, these studies underline that the protective effects of vitD3 supplementation are most likely affected by baseline vitD3 status as well as dosing frequency.

Conclusion

Our results suggest that a physiological dosing schedule based on daily supplementation with vitD3 in combination with PBA can be used to ameliorate clinical symptoms and TB-specific AEs, primarily in vitD3-deficient TB patients. Therefore, although vitD3 + PBA may not be applicable as a therapeutic intervention to a broad range of TB patients, supplementation may turn out promising for certain high-risk groups with vitD3 deficiency, immunodeficiency diseases, MDR-TB or latent TB. In contrast to treatment of active TB, there is a possibility that nutritional supplementation will have a greater impact on the prevention of disease among individuals with latent TB and vitD3 deficiency [41]. Such prophylactic studies are complicated to implement, but would shed additional light on the potential benefit of vitD3 + PBA immunotherapy.

Acknowledgements

We thank the local study team at the Black Lion Hospital and the Armauer Hansen Research Institute (AHRI), as well as all the nurses and administrative staff at the collaborative health centres in Addis Ababa, Ethiopia. We also thank the members of our Data Monitoring and Safety Board. Finally, we would like to sincerely thank all the patients who participated in this trial. We also thank BioScience Writers (BSW) in Houston, Texas, for assistance with language editing of this manuscript.

Funding

This study was funded by the Swedish Contingency Agency (MSB) and the Swedish International Development Agency (Sida) (2010-7938), the Swedish Research Council (VR) (K2015-56X-20665-08-3), the Swedish Heart and Lung Foundation (HLF) (20140752) and Karolinska Institutet (senior research position of SB). Merck Serono kindly donated Vigantoletten and placebo tablets and also assisted the study with labels. NB was supported in part by the National Institute of Health (NIH) Research Training grant R25TW009337, funded by the Fogarty International Center, the NIH Office of the Director, and the National Institute of Mental Health.

Conflict of interest statement

No conflict of interest to declare.

Author’s contributions

S.B., P.B., J.A., S.A., A.B., E.K., W.A., G.Ad., B.A. and R.R. contributed to study design. S.B., P.B. and J.A. wrote the protocol. S.B. acquired funding. S.B., S.A., N.G. and A.B. acquired ethics permissions. N.G., A.B., S.A., M.T., E.K., W.A. and G.Ad. coordinated the clinical work, collected data and participated in data management. G.As. read chest radiographs. N.G. and A.S. coordinated the laboratory analyses. S.B., N.G., S.A. and G.Ad. supervised data collection. S.B., P.B., J.A. participated in data analysis, and data interpretation. U.H. and A.W. performed the statistical analyses. S.B. wrote the manuscript and did the literature search; all other authors critically reviewed the content and approved the final version.

External editor

The manuscript has been handled by an external editor, Professor of Medicine Sam Schulman, Thrombosis Service, McMaster Clinic & HHS – General Hospital, Canada.

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Supporting Information
Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Methods.

**Figure S1.** Distribution of the primary TB score.

**Figure S2.** Radiological findings.

**Table S1.** Modified clinical TB score.

**Table S2.** Subgroup analyses: clinical TB score in vitD3 + PBA versus placebo.

**Table S3.** Laboratory adverse events.

**Table S4.** Blood chemistry data.