Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment

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Abstract

Calcidiol, the major circulating metabolite of vitamin D, supports induction of pleiotropic antimicrobial responses *in vitro*. Vitamin D supplementation elevates circulating calcidiol concentrations, and thus has a potential role in the prevention and treatment of infection. The immunomodulatory effects of administering vitamin D to humans with an infectious disease have not previously been reported. In order to characterise these effects, we conducted a detailed longitudinal study of circulating and antigen-stimulated immune responses in ninety-five patients receiving antimicrobial therapy for pulmonary tuberculosis who were randomised to receive adjunctive high-dose vitamin D or placebo in a clinical trial, and who fulfilled criteria for per-protocol analysis. Vitamin D supplementation accelerated sputum smear conversion and enhanced treatment-induced resolution of lymphopaenia, monocytosis, hypercytokinaemia and hyperchemokinaemia. Administration of vitamin D also suppressed antigen-stimulated proinflammatory cytokine responses, but attenuated the suppressive effect of antimicrobial therapy on antigen-stimulated secretion of interleukin-4, CC chemokine ligand 5 and interferon-α. We demonstrate a previously unappreciated role for vitamin D supplementation in accelerating resolution of inflammatory responses during tuberculosis treatment. Our findings suggest a potential role for adjunctive vitamin D supplementation in the treatment of pulmonary infections to accelerate resolution of inflammatory responses associated with increased risk of mortality.

Introduction

Despite the widespread availability of antimicrobials, bacterial respiratory infections remain a major global cause of death(1). Mortality is associated with infection with antibiotic-resistant organisms(2, 3) and with failure to resolve immunopathological inflammatory responses(4-6). Immunomodulatory agents that augment antimicrobial immune responses and accelerate resolution of pulmonary inflammation could be used as adjuncts to antimicrobial therapy to improve treatment outcomes(7).

Calcitriol, the active metabolite of vitamin D, induces innate antimicrobial responses and suppresses pro-inflammatory cytokine responses *in vitro*(8). Its antimicrobial activity is mediated *via* induction of reactive nitrogen intermediates, reactive oxygen intermediates, antimicrobial peptides and autophagy(9). Calcitriol also modulates adaptive responses, both indirectly (by suppression of MHC class II expression and IL-12 secretion by antigen-presenting cells) and directly (by suppressing secretion of interferon-γ (IFN-γ) and interleukin-2 (IL-2) from CD4+ T helper type 1 (Th1) cells(10)). Calcitriol is synthesised by the vitamin D 1-alpha hydroxylase enzyme, whose expression is upregulated in leucocytes and pulmonary epithelium following ligation of toll-like receptors by pathogen-associated ligands(11, 12). Extra-renal generation of calcitriol is dependent on the availability of its precursor calcidiol, the major circulating vitamin D metabolite that supports induction of antimicrobial responses *in vitro*(11, 13) and whose concentrations are often low in patients with pulmonary infection(14-16). Vitamin D supplementation elevates circulating calcidiol concentrations, and may therefore enhance response to antimicrobial therapy for respiratory infections. However, the effects of *in vivo* vitamin D supplementation on immune responses in humans with an infectious disease have not previously been described.

Vitamin D was used to treat tuberculosis in the pre-antibiotic era(17), and vitamin D supplementation has been shown to enhance healthy tuberculosis contacts' immunity to mycobacteria(18). These observations prompted us to conduct a randomised controlled trial evaluating the influence of adjunctive high-dose vitamin D on time to bacterial clearance in patients receiving antimicrobial therapy for smear-positive pulmonary tuberculosis(19). We now present results of a detailed analysis of longitudinal changes in the immune response in trial participants during the 8-week course of intensive-phase antituberculous therapy. Initially we describe the effects of antituberculous therapy alone on circulating and antigen-stimulated immune responses, using samples from patients randomised to the placebo arm of the trial. Subsequently we proceed to characterise the effects of vitamin D

supplementation using samples from those randomised to the intervention arm of the study, showing that administration of adjunctive vitamin D exerts pleiotropic immunomodulatory effects in patients with pulmonary tuberculosis.

Results

Effect of antimicrobials on circulating responses

Determination of the immunomodulatory effects of vitamin D supplementation necessitated an initial, comprehensive characterisation of the changes in immune responses induced by antituberculous therapy alone. To this end, 42 soluble factors and 14 haematological parameters detailed in Materials and Methods were measured in samples of serum, plasma and whole blood taken from 51 patients randomised to the placebo arm of the trial at 0, 2, 4, 6 and 8 weeks of treatment (for trial profile see Figure S1; for baseline characteristics, see Table S1). Parameters were selected on the basis that they played a role in host defence against *Mycobacterium tuberculosis* (MTB)(20) and / or that they were biomarkers of treatment response (21). Median serum concentrations of seven soluble factors (IL-2, IL-5, IL-13, IL-17, tumour necrosis factor (TNF), basic fibroblast growth factor (FGF-β) and matrix metalloproteinase-7 (MMP-7) were below the limit of detection (LOD) at baseline, and these were excluded from statistical analyses. The remaining 49 parameters were assessed using principal component analysis (PCA), a well-established mathematical technique for reducing the dimensionality of complex data sets by transformation of data to a 3-dimensional coordinate system (22). The resultant 3-dimensional PCA plot (Video S1A) showed a gradient of samples from week 0 to 8 along principal component 1. Baseline samples occupied more space than follow-up samples (13/51 vs. 30/49 data points with Euclidean distance <5 at baseline vs. 8 weeks respectively, p=0.003), indicating that patients had a relatively heterogeneous immunological profile at baseline that became more homogenous as treatment progressed.

Rank regression analysis (23) was applied to PCA-transformed data in order to identify parameters whose concentration changed significantly over time. Table 1 presents details of the 42 circulating immunological parameters so identified. Of the haematological parameters investigated, platelet count, neutrophil count and monocyte count decreased during the course of treatment ($p \leq 0.0018$), while lymphocyte count and eosinophil count both increased ($p \leq 0.0019$). Increases were also seen in haemoglobin concentration and red blood cell parameters ($p \leq 0.015$), reflecting resolution of microcytic anaemia as treatment progressed. Decreases in erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) ($p < 1.24 \times 10^{-12}$) and an increase in serum albumin concentration ($p = 5.17 \times 10^{-21}$) were also seen, indicating resolution of the acute phase response. These changes were accompanied by a decrease in circulating concentrations of all cytokines, chemokines, antimicrobial

peptides (AMP), MMP and angiogenic factors identified ($p \leq 0.0240$) except for CCL2 and MMP-2, whose concentration increased during the course of treatment ($p \leq 0.0035$).

To investigate the relationship between changes in cell counts and circulating concentrations of inflammatory mediators observed during treatment, network PCA was applied to the parameters listed in Table 1. Seven distinct clusters were identified (Video S2A). For parameters whose values fell during treatment, the tightest cluster incorporated three MMP (MMP-1, MMP-8 and MMP-9) with three AMP (human neutrophil peptides [HNP] 1-3, neutrophil gelatinase-associated lipocalin [NGAL] and cathelicidin [LL-37]); this cluster was close to neutrophils, CXCL8 and PGE2. Neutrophils were linked to monocytes and CRP, which in turn was linked to IL-6 and ESR. Platelet count and CCL5 formed a distinct grouping, while the other IFN-γ-stimulated chemokines CXCL9 and CXCL10 were linked to each other and to IFN-γ, which was linked to IL-6. The angiogenic factors epidermal growth factor (EGF) hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) were linked to each other and IL-7, IL-10, IL-15 and soluble IL-2 receptor (IL-2R). For parameters increasing during treatment, one network incorporated red blood cell parameters, albumin and MMP-2, and another linked lymphocyte and eosinophil count.

Effect of antimicrobials on antigen-stimulated responses

Whole blood samples taken from 28 patients randomised to the placebo arm of the trial were stimulated *ex vivo* with a panel of mycobacterial antigens, and the concentration of IFN-γ in supernatants of baseline samples was compared between stimuli. Of the MTB-specific antigens tested, recombinant early secreted antigenic target, 6 kDa, (rESAT-6) and recombinant culture filtrate protein, 10 kDa (rCFP-10) induced the greatest IFN-γ responses (Figure S2). We therefore proceeded to assay concentrations of 39 soluble factors listed in Materials and Methods in supernatants of whole blood stimulated with these two antigens at 0, 2, 4, 6 and 8 weeks of treatment. Median concentrations of six soluble factors (IL-2, IL-5, IL-13, EGF, FGF-β and MMP-7) were below the LOD in these samples, and these parameters were therefore excluded from statistical analyses. The remaining 33 parameters were analysed by PCA and rank regression. The resultant PCA plots for rESAT-6- and rCFP-10-stimulated responses (Video S1B-C) were similar to each other: as before, samples converged from a loosely clustered pattern towards a more tightly clustered pattern as treatment progressed. Twenty-seven antigen-stimulated parameters contributed to the pattern of response to antituberculous therapy (Table 1). All analytes whose concentration changed significantly during the course of intensive phase antituberculous therapy showed a decrease in secretion over time. Of note, IFN-γ was among the analytes whose antigen-

stimulated concentration did not change significantly during the course of antituberculous therapy, even when corrected for changes in lymphocyte count (Figure S3).

In order to determine whether changes in antigen-stimulated immune responses corresponded to changes in whole blood cellular composition during antituberculous therapy, network PCA was applied to the antigen-stimulated analytes listed in Table 1 together with cell count data obtained for the relevant samples prior to antigenic stimulation. Similar PCA networks were identified for rCFP-10- and rESAT-6-stimulated responses (Video S2B-C). Platelets, CCL5, IL-4, granulocyte-colony stimulating factor (G-CSF) and CCL11 were connected in both plots, replicating the platelet-CCL5 connection observed in the analysis of circulating parameters (Video S2A). Neutrophils occupied a similar space to neutrophil granule-associated proteins MMP-9 and NGAL. IL-7 clustered with another angiogenic factor, VEGF, and Th1 cytokines. Lymphocytes were not connected directly to any cytokines stimulated by rESAT-6 or rCFP-10.

Effects of vitamin D on circulating responses

We have previously reported results of the intention-to-treat analysis of study data, indicating that administration of adjunctive vitamin D was associated with a trend towards faster sputum culture conversion (p=0.14)(19). We repeated this analysis in the subgroup of 95 participants fulfilling per-protocol analysis criteria, adjusting for factors previously shown to influence time to sputum conversion in this dataset (age, ethnicity, baseline sputum smear, neutrophil count, and presence or absence of cavitation on baseline chest radiograph). Median time to sputum culture conversion in this subset of patients was 35.0 days in the intervention group and 46.5 days in the control group (hazard ratio 1.27, 95% CI 0.76 to 2.13, p=0.36), while median time to sputum smear conversion in the intervention arm was significantly shorter than that in the control arm (23 *vs.* 36 days; hazard ratio 1.69, 95% CI 1.02 to 2.79, p=0.04; Figure 1A).

In order to determine whether the effect of vitamin D on time to sputum clearance in the per-protocol sub-group was associated with immunomodulatory activity, we compared the effect of antituberculous therapy on the circulating immunological parameters investigated above in 51 patients randomised to the placebo arm of the trial *vs.* 44 patients randomised to receive adjunctive vitamin D using PCA and rank regression on the interaction term ‘treatment duration*allocation’. The PCA plot is shown in Video S3A. The seventeen circulating parameters identified as being significantly affected by vitamin D are detailed in Table 2. All of these were also significantly affected by intensive phase antituberculous therapy: in every

case, vitamin D accelerated the effect of antituberculous therapy. The analyte most affected by vitamin D was the chemokine CXCL9, whose serum concentration decreased significantly faster in patients randomised to receive vitamin D *vs.* placebo ($p=5.92 \times 10^{-12}$). Serum concentrations of three other chemokines (CXCL10, CCL3 and CCL5) also fell more rapidly in patients randomised to vitamin D *vs.* placebo ($p \leq 0.0164$), as did IFN-γ ($p=0.0012$). Monocyte counts fell more rapidly ($p=3.54 \times 10^{-4}$) and lymphocyte counts rose more rapidly ($p=0.0365$) among patients receiving vitamin D. Neutrophil counts were not significantly affected by allocation, but plasma concentrations of neutrophil-associated AMP (LL-37, HNP1-3 and NGAL) and MMP-9 fell more quickly in patients receiving adjunctive vitamin D ($p \leq 0.0111$). Administration of vitamin D also induced a more rapid drop in ESR and serum CRP concentration ($p \leq 0.0027$), indicating accelerated resolution of the acute phase response.

Network PCA indicated that the accelerated fall in monocyte count in patients receiving vitamin D was linked to a rise in lymphocyte count and a decrease in ESR and serum CRP concentration (Figure S4A). The IFN-γ-inducible chemokines CXCL9 and CXCL10 were linked to IFN-γ, IL-2R, IL-10 and CCL3. NGAL, HNP1-3 and MMP-9 formed another cluster, which was linked to LL-37 and PGE2. The kinetics of change in a representative group of circulating immunological parameters over the course of intensive phase therapy in vitamin D *vs.* placebo arms are presented in Figure 1B-H.

We have previously reported that the effect of vitamin D on time to sputum culture conversion is modified by the *TaqI* genotype of the vitamin D receptor (VDR), such that vitamin D hastens sputum culture conversion in patients with the *tt* genotype, but not in those with *Tt* or *TT* genotypes (Figure S5A-C)(19). To investigate whether the effects of vitamin D on circulating immune responses were also restricted to patients with this genotype, we stratified analysis of the effects of vitamin D on 8-week values of analytes listed in Table 2 according to *TaqI* genotype. In patients with the *TT* genotype, vitamin D supplementation significantly reduced 8-week circulating concentrations of CRP, CXCL9, CXCL10, NGAL and LL-37, while in those with the *Tt* genotype, vitamin D significantly reduced 8-week circulating concentrations of CXCL9 and IL-10 (Figure S5D-I; $p \leq 0.045$). These analyses indicate that immunomodulatory effects of vitamin D are not restricted to individuals with the *tt* genotype of the *TaqI* VDR polymorphism.

Effects of vitamin D on antigen-stimulated responses

We next investigated the effect of vitamin D supplementation on antigen-stimulated responses using PCA and interaction analysis on 34 analytes detailed above for 19 patients allocated to vitamin D *vs.* 28 patients allocated to placebo. The PCA plots generated for responses to rESAT-6 and rCFP-10 were similar to each other, and samples from patients allocated to vitamin D *vs.* placebo were clearly separated (Video S3B-C). Vitamin D supplementation influenced supernatant concentrations of seven analytes in both rESAT-6- and rCFP-10-stimulated whole blood (Table 2); notably, IFN-γ was not among them (Figure S3). Vitamin D enhanced the suppressive effect of antimicrobial therapy on secretion of IL-1 receptor antagonist (IL-1RA), IL-6, IL-12 and TNF ($p \leq 0.0437$) and attenuated treatment-induced reductions in secretion of IL-4, CCL5 and IFN-α ($p \leq 0.0323$). Network PCA showed that IL-12, TNF and IL-1RA were linked, while IL-6 was connected to monocytes directly or via VEGF, and CCL5 and IL-4 were tightly clustered and linked to IFN-α for both antigens (Figure S4B-C). Vitamin D significantly reduced antigen-stimulated secretion of IL-12, TNF, IL-1RA, IL-6, CXCL10, CCL3, CCL4 and VEGF (Figure 1I-L) and enhanced CCL5, CCL11, IL-4 and IFN-α secretion.

Discussion

Our study represents the most detailed characterisation of the effects of antituberculous therapy on the immune response conducted to date, and the first clinical investigation into the immunomodulatory actions of *in vivo* vitamin D supplementation during treatment of an infectious disease. In patients taking antimicrobial therapy for smear-positive pulmonary tuberculosis, adjunctive vitamin D accelerated sputum smear conversion, augmented treatment-induced increases in lymphocyte count and enhanced the suppressive effect of treatment on monocyte count, inflammatory markers and circulating concentrations of chemokines, antimicrobial peptides and MMP-9. Administration of vitamin D also enhanced treatment-induced suppression of antigen-stimulated Th1 cytokine responses, but attenuated treatment-induced suppression of antigen-stimulated IL-4, CCL5 and IFN-α secretion.

Among 51 patients randomised to receive antituberculous therapy plus placebo, we observed an increase in circulating lymphocyte count and a reduction in circulating neutrophil counts, monocyte counts and concentrations of IFN-inducible parameters following initiation of antituberculous therapy, consistent with previous reports (24-26). These changes were associated with decreases in circulating concentrations of lymphocyte chemoattractants CXCL9 and CXCL10, and an increase in the monocyte chemoattractant CCL2; they may therefore reflect reduced recruitment of lymphocytes, and increased recruitment of monocytes, to the lung. In keeping with this hypothesis, the proportion of macrophages in sputum of tuberculosis patients has been reported to increase as treatment progresses (27). Interestingly, increases in circulating lymphocyte count following initiation of antituberculous therapy were not associated with any change in antigen-stimulated production of IFN-γ as treatment progressed. By contrast, antigen-stimulated production of CCL5, IL-4, G-CSF, IFN-α and CXCL10 were greatly decreased over the course of intensive-phase therapy. Antigen-stimulated CXCL10 responses have been reported to be more sensitive than IFN-γ for the diagnosis of active tuberculosis(28), and these data suggest that this panel of analytes may also hold promise as antigen-stimulated biomarkers of treatment response. Resolution of thrombocytosis is another well recognised phenomenon associated with TB treatment(29), and network analysis revealed this to be linked to a decrease in circulating CCL5 and antigen-stimulated CCL5 and IL-4 among patients in our study. Although best known for their role in haemostasis, platelets are also recognised to secrete CCL5(30), which can enhance production of IL-4 by CD4+ T cells(31). The role of platelets in the antimycobacterial response warrants further investigation.

Having characterised the immune response to antituberculous therapy, we proceeded to investigate how this was affected by administration of adjunctive vitamin D. In contrast to studies investigating immunomodulatory actions of vitamin D supplementation in healthy people and in those with non-communicable diseases (32-35), we report pleiotropic immunomodulatory actions of vitamin D in tuberculosis patients. This difference may reflect the very high prevalence of profound deficiency at baseline among participants in our study; the relatively high dose of vitamin D administered; or the fact that MTB can upregulate expression of the vitamin D 1-alpha hydroxylase CYP27B1 to generate immunomodulatory concentrations of calcitriol at sites of infection(11). Among patients fulfilling criteria for per-protocol analysis (n=96), vitamin D accelerated sputum smear conversion (p=0.04). This finding contrasts with results of our previously published intention-to-treat analysis (n=126), in which a trend towards faster conversion in vitamin D-supplemented patients did not attain statistical significance (19). This difference may reflect the superior compliance of participants included in the per-protocol analysis, which excluded patients who did not take a full course of study medication. Vitamin D also suppressed circulating concentrations of IFN-γ and IFN-γ-inducible chemokines CXCL9 and CXCL10, the matrix metalloproteinase enzyme MMP-9 and antigen-stimulated Th1 responses. These *in vivo* findings are consistent with reported immunomodulatory actions of calcitriol *in vitro* (8, 36, 37). In contrast to these suppressive actions, vitamin D also attenuated treatment-induced falls in antigen-stimulated CCL5, IL-4 and IFNα. IL-4 has recently been reported to induce expression of CYP24A, the principal catabolic enzyme of both calcidiol and calcitriol(38); the increase in antigen-stimulated IL-4 secretion observed in the intervention arm of the study may therefore represent part of a negative feedback loop *via* which calcitriol regulates its own concentration at the site of disease. The finding that administration of vitamin D enhanced antigen-stimulated IFN-α responses is of particular interest, given the pivotal role of type 1 interferons in antiviral responses(39), and the clinical observation of a 6-fold reduction in upper respiratory tract infections among patients in the intervention arm of the trial(19). Modulation of antigen-stimulated responses by vitamin D supplementation may represent changes in numbers of circulating lymphocyte sub-populations and / or direct effects of vitamin D on lymphocyte function. More detailed characterisation of the effects of vitamin D supplementation on numbers and cytokine profiles of lymphocyte subsets is warranted.

Although many of the immunomodulatory effects of *in vivo* vitamin D supplementation that we observed were in keeping with the *in vitro* actions of calcitriol, there were two exceptions: calcitriol has been reported to induce IL-10(36) and the antimicrobial peptides LL-37 and NGAL(40, 41) *in vitro*, but we found that *in vivo* vitamin D supplementation suppressed circulating concentrations of IL-10, LL-37 and NGAL. All three of these markers are

suppressed by antituberculous therapy alone (Table 1), and the fact their concentration fell more quickly among patients in the intervention arm of the study may arise as an indirect consequence of enhanced microbial killing in patients receiving vitamin D. Alternatively, this observation may represent a direct suppressive effect of vitamin D on release of these mediators into the circulation from neutrophil granules.

Interestingly, and in contrast to the effects of vitamin D supplementation on sputum clearance that we have previously demonstrated (19), we found that immunomodulatory effects of vitamin D were observed in patients having the TT and Tt genotypes of the *TaqI* vitamin D receptor polymorphism (the effects of vitamin D in patients with the tt genotype could not be determined, due to small numbers in this group). This observation suggests that if these responses can be augmented - by administering vitamin D at higher doses, for example - then tuberculosis patients might derive a clinical benefit from vitamin D supplementation irrespective of *TaqI* genotype. More broadly, the ability of vitamin D to accelerate resolution of potentially immunopathological inflammatory responses without compromising bacterial killing raises the possibility that supplementation might also have benefits in patients receiving antimicrobial therapy for pneumonia and sepsis, in whom failure to resolve hypercytokinaemia is associated with increased mortality(5, 6).

Materials and Methods

Details of the trial protocol have previously been reported; participants were randomised to receive four fortnightly doses of 2.5 mg vitamin D_3 *vs.* placebo in addition to standard antituberculous therapy(19). Antigen-stimulated whole blood assays were performed as previously described(42). Concentrations of IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, G-CSF, GM-CSF, IFN-α, IFN-γ, TNF, CXCL8, CXCL9, CXCL10, CCL2, CCL3, CCL4, CCL5, CCL11, EGF, FGF-β, HGF and VEGF were quantified using a human 30-plex bead immunoassay panel (Invitrogen, Camarillo, CA, USA). Serum CRP and albumin concentrations were assayed using an Architect ci8200 analyser (Abbott Diagnostics, Chicago, IL, USA). Serum PGE2 concentration was analysed by high sensitivity competitive enzyme immunoassay (Assay Designs, Ann Arbour, MI, USA). Concentrations of LL-37, HNP1-3 and NGAL were analysed by ELISA (Hycult Biotechnology, Uden, The Netherlands). Concentrations of MMP-1, -2, -3, -7 and -8 were determine by Fluorokine MAP multianlalyte profiling (R&D systems); concentration of MMP-9 was determined by DuoSet ELISA (R&D systems). Antigen-stimulated AMP and MMP concentrations were corrected by subtraction of unstimulated values. Full blood counts were performed using a LH750 haematology analyser (Beckman Coulter, Brea, CA, USA). ESR was measured by the Wintrobe method using a s2000 analyser (Desaga, Wiseloch, Germany). DNA extraction and genotyping were performed as previously described (19).

PCA was conducted using Qlucore Omics Explorer 2.2 (Qlucore AB, Lund, Sweden). Analyte concentrations were $\log_2$ converted and normalised to the mean for each analyte with variance -1 to +1. PCA networks were created using 1 connection. Rank regression analysis was applied to PCA-transformed data to identify parameters whose concentration was affected by antituberculous therapy (by making within-patient comparison of samples at different timepoints among patients allocated to placebo) and vitamin D (by making between-patient comparison of samples from patients allocated to placebo vs. vitamin D.) These analyses yielded t statistics (calculated as the regression co-efficient for each parameter divided by its standard deviation) representing the magnitude of difference in concentration of a given parameter between groups being compared, and p values, representing the probability that such differences could have arisen by chance alone. The effects of allocation on circulating immune responses at 8 weeks within genetically defined sub-groups were analysed using Mann-Whitney U tests. The effect of allocation on time to sputum clearance was analysed by Cox regression analysis, The effect of allocation on time to sputum clearance was analysed by Cox regression analysis, adjusting for age, ethnicity, baseline

sputum smear, neutrophil count, and presence or absence of cavitation on baseline chest radiograph as previously described (19).

Further details of materials and methods are presented in Text S1.

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Figure legends

Figure 1. Kinetics of circulating and antigen-stimulated immune responses during the course of antituberculous therapy in the presence *vs.* the absence of adjunctive vitamin D. Vitamin D accelerated sputum smear conversion in patients fulfilling per-protocol analysis criteria (A). Monocyte counts fell more quickly (B) and lymphocyte counts rose more quickly (C) among patients in the intervention arm of the trial. Vitamin D also accelerated treatment-induced decreases in erythrocyte sedimentation rate (ESR, D), circulating concentrations of CXCL9 (E), CXCL10 (F), MMP-9 (G) and LL-37 (H) and rCFP-10-stimulated supernatant concentrations of IL-1RA (I), IL-6 (J), IL-12p40/p70 (K) and TNF (L). Means ± SEM at 0, 2, 4, 6 and 8 weeks of treatment are presented. Dotted lines, placebo arm; solid lines, vitamin D arm.

Table legends

Table 1. Influence of intensive-phase antituberculous therapy on circulating and antigen-stimulated immune responses in adults with smear-positive pulmonary tuberculosis

Table 2. Influence of adjunctive vitamin D supplementation on circulating and antigen-stimulated immune responses to intensive-phase antituberculous therapy