Paradoxical upgrading reaction in extra-pulmonary tuberculosis: association with vitamin D therapy

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SUMMARY

SETTING: Glasgow, Scotland, UK.

BACKGROUND: Paradoxical reactions in tuberculosis (TB) are a notable example of our incomplete understanding of host-pathogen interactions during anti-tuberculosis treatment.

OBJECTIVES: To determine risk factors for a TB paradoxical reaction, and specifically to assess for an independent association with vitamin D use.

DESIGN: Consecutive human immunodeficiency virus (HIV) negative adult patients treated for extra-pulmonary TB were identified from an Extended Surveillance of Mycobacterial Infections database. In our setting, vitamin D was variably prescribed for newly diagnosed TB patients. A previously published definition of paradoxical TB reaction was retrospectively applied to, and data on all previously described risk factors were extracted from, centralised electronic patient records. The association with vitamin D use was assessed using multivariate logistic regression.

RESULTS: Of the 249 patients included, most had TB adenopathy; 222/249 had microbiologically and/or histologically confirmed TB. Vitamin D was prescribed for 57/249 (23%) patients; 37/249 (15%) were classified as having paradoxical reactions. Younger age, acid-fast bacilli-positive invasive samples, multiple disease sites, lower lymphocyte count and vitamin D use were found to be independent risk factors.

CONCLUSION: We speculate that vitamin D-mediated signalling of pro-inflammatory innate immune cells, along with high antigenic load, may mediate paradoxical reactions in anti-tuberculosis treatment.

KEY WORDS: host-directed therapy; innate immunity; host–pathogen interaction; inflammation

WORSENING OF TUBERCULOSIS (TB) disease despite receipt of effective anti-tuberculosis treatment is referred to as a ‘paradoxical upgrading reaction’ (PUR). A PUR is a clinical diagnosis based on worsening of an existing TB lesion or development of newly apparent TB lesions, which are typically culture-negative and not associated with treatment failure.1–7 PURs are most frequently diagnosed at extra-pulmonary sites, where they can cause significant morbidity. In addition, imaging modalities such as positron emission tomography-computed tomography reveal that most pulmonary TB (PTB) patients have new lesions or lesions with increased metabolic activity after 6 months of anti-tuberculosis treatment despite sputum culture conversion.8 Similar rates of new, subclinical lesions are seen on serial magnetic resonance imaging of the brains of patients after receipt of treatment for central nervous system TB.9 Rather than being an unusual event, PURs may be an underappreciated central feature of the interaction between Mycobacterium tuberculosis, host immunity and antimicrobial treatment.

Historically, PURs have been thought to be analogous to ‘upgrading reactions’ in leprosy.10 More recently, TB PUR in the context of human immunodeficiency virus (HIV) associated immune reconstitution inflammatory syndrome (TB-IRIS) has been described. Pathogenesis of antiretroviral treatment-associated IRIS is increasingly understood to involve innate immune mediators, including Toll-like receptor (TLR) signalling.11

How a PUR might develop in the absence of overt reversal of immune suppression as observed in HIV-associated IRIS is not clear. A small number of studies have indicated extra-pulmonary TB (EPTB), baseline lymphopaenia and increased peripheral lymphocyte
reconstitution to be risk factors in non-HIV-infected TB patients. Vitamin D is a known immune modulator in TB infection, and vitamin D deficiency has been associated with PURs in some case reports. No studies have assessed vitamin D supplementation as a modulator of PUR risk. A randomised control trial of high-dose vitamin D supplementation during the intensive phase of PTB treatment reported a PUR in 2/71 in the intervention group and 0/70 in the placebo group—a non-significant difference. However, that study had 56 days of follow-up (less than the median time to a PUR in most studies) and was not powered to detect PUR outcomes. A potential association between use of vitamin D and PURs is therefore a pressing but open question.

Prescription of vitamin D is increasingly common practice in TB clinics in our setting. This gave us an opportunity to carry out a retrospective cohort study to examine the effect of vitamin D use on the risk of symptomatic PURs in patients treated for EPTB.

METHODS

Patients treated for EPTB at four hospitals collectively responsible for >95% of TB management in the Greater Glasgow and Clyde area of Scotland were included in a retrospective cohort. In this setting, vitamin D was prescribed ad hoc such that patients received vitamin D at the discretion of individual treating clinicians. Included were all consecutive patients who: 1) were aged ≥18 years; 2) had standard therapy for confirmed EPTB (culture-positive or positive acid-fast bacilli [AFB] history or smear) or probable EPTB (clinical, histological or radiological evidence of TB with response to anti-tuberculosis treatment); and 3) were HIV-negative. Patients already prescribed vitamin D before the diagnosis of TB, and those with <3 months of recorded follow-up, were excluded.

Patients were identified from an Extended Surveillance of Mycobacterial Infections database to which all TB cases in Glasgow are notified. Demographic and clinical data were obtained from centralised electronic patient records and patient folders as necessary. Independent variables collected included all previously published risk factors for a PUR, and any prescription of a vitamin D supplement during TB treatment as a binary variable (for variable definitions and prior literature review, see the Appendix). This retrospective review of routinely collected data was exempted from formal ethics review.

A published definition of a PUR (‘worsening of pre-existing tuberculous lesions on the basis of clinical or radiological findings or development of new TB lesions in patients who had received antituberculosis treatment for at least 10 days and whose conditions were reported to be improving’) was retrospectively applied to all cases by two consultants in infectious diseases (RAS and DB) blinded to each other’s assessment and the status of vitamin D prescription. In cases of disagreement, a third independent application of the case definition (DAB) was used to break ties.

Assuming vitamin D was prescribed in 50% of EPTB patients with an overall PUR prevalence of 18%, a sample size of 250 patients was calculated to give 0.80 power at α = 0.05 to detect a 12% absolute increase in PURs associated with vitamin D use. Pairwise comparison of variables was by Fisher’s exact test for categorical variables, and Wilcoxon’s rank-sum test for non-normal numerical variables. The association of vitamin D use with a PUR after adjustment for variables thought to be potential confounders was assessed by logistic regression. All statistical analyses were carried out in R Studio v0.99.902 (R Computing, Vienna, Austria); R code to reproduce this analysis is available at https://github.com/davidadambarr/PUR.EPTB.VitD.

RESULTS

Description of cohort

Of 260 patients included, 249 were assessed; 11 were excluded due to evidence of vitamin D prescription before a TB diagnosis (n = 5) or due to <3 months of recorded follow-up (n = 6). Basic demographic and clinical descriptors are shown in Table 1. Most patients were of South Asian ethnicity; lymph nodes were the most common site of disease. Invasive diagnostic sampling (e.g., biopsy or aspiration) was attempted in 230 patients (92%); 153/230 (67%) were M. tuberculosis culture-positive; 69/230 (30%) were culture-negative but AFB-positive or had histological features in keeping with TB disease.

Of the 93 patients (37%) who had a baseline serum level of 25-hydroxycholecalciferol checked, 45/93 (48%) had levels below the limit of detection for the assay (<7 nmol/l, range <7 to 114 nmol/l). Vitamin D was prescribed for 57 patients (23%), not necessarily according to baseline status, because not all patients prescribed vitamin D had a baseline level checked. For 52 (91%) of these patients, a dose equivalent to ≤800 international units (IU) of cholecalciferol per day was used, and 5/57 (9%) received a one-off dose of 300,000 IU, followed by 20,000 IU monthly. Prescription of vitamin D differed according to patient ethnicity, the clinic at which TB was being treated, and by the baseline level of vitamin D, although vitamin D deficiency was also prevalent among patients not prescribed vitamin D (Table 1).
Thirty-seven patients (15%) were classified as having a PUR. Inter-rater agreement was high for the two primary assessors (Cohen's \( \kappa \) 0.84; 95% confidence interval [CI] 0.74–0.94). The time of the first PUR after starting anti-tuberculosis treatment had a positively skewed distribution (median 52 [range 10–500] days). Corticosteroid treatment was prescribed for 14/37 (38%) patients after a PUR; 5/37 (14%) had percutaneous drainage; 12/37 required no specific treatment; and one patient had surgical intervention for constrictive pericarditis associated with a PUR.

Of 249 patients, 241 (97%) had a recorded outcome available at the end of the treatment: 239/241 (99%) were recorded as ‘clinically cured’, the remaining two died on treatment (neither thought to be related to a PUR). A median post-treatment follow-up of 12 months was recorded; 3/239 (1%) patients had recorded recurrent/relapsed TB at respectively 2, 4 and 24 months after the end of treatment.

### Table 1: Description of cohort

| Overall (\( n = 249 \)) | Vitamin D not prescribed (\( n = 192 \)) | Vitamin D prescribed (\( n = 57 \)) | P value* |
|--------------------------|------------------------------------------|-------------------------------------|---------|
| Age, years, median [IQR] | 36 [28.9–50.7] | 38.7 [29.5–52.3] | 32.7 [26.8–43.4] | 0.055 |
| Female | 91 (36.5) | 69 (35.9) | 22 (38.6) | 0.755 |
| Paradoxical upgrading reaction | 37 (14.9) | 20 (10.4) | 17 (29.8) | 0.001 |
| Ethnicity | | | | 0.039 |
| African | 27 (10.8) | 18 (9.4) | 9 (15.8) | |
| Middle Eastern | 2 (0.8) | 1 (0.5) | 1 (1.8) | |
| South East Asian | 11 (4.4) | 10 (5.2) | 1 (1.8) | |
| South Asian | 149 (59.8) | 110 (57.3) | 39 (68.4) | |
| White European | 60 (24.1) | 53 (27.6) | 7 (12.3) | |
| Clinic site | | | | <0.001 |
| A | 104 (41.8) | 97 (50.5) | 7 (12.3) | 1 |
| B | 62 (24.9) | 38 (19.8) | 24 (42.1) | 1 |
| C | 53 (21.3) | 42 (21.9) | 11 (19.3) | 1 |
| D | 30 (12.0) | 15 (7.8) | 15 (26.3) | 1 |
| Baseline blood results, median [IQR]^† | | | | |
| Lymphocytes \( \times 10^9/l \) | 1.4 [1.02–1.90] | 1.4 [1.04–1.90] | 1.34 [0.97–1.82] | 0.592 |
| Monocytes \( \times 10^9/l \) | 0.6 [0.43–0.80] | 0.61 [0.50–0.80] | 0.56 [0.40–0.80] | 0.177 |
| Neutrophils \( \times 10^9/l \) | 4.5 [3.40–6.08] | 4.55 [3.40–5.90] | 4.41 [3.38–6.78] | 0.665 |
| Haemoglobin \( \times 10^9/l \) | 130 [115–140] | 130 [116–141] | 125 [111–137] | 0.223 |
| ESR, mm/h | 29 [13–51] | 33 [13–50] | 27 [10–61] | 0.944 |
| Albumin, g/l | 34 [30–38] | 35 [30–38] | 33 [28–37] | 0.131 |
| CRP, mg/l | 25 [5–65] | 21 [5–65] | 29 [6–65] | 0.505 |
| 25-hydroxycholecalciferol, nmol/l | 10 [LDL–19.0] | 16 [LDL–26.0] | LDL [LDL–10.0] | 0.004 |
| Sites of TB disease | | | | |
| Pleural | 48 (19.3) | 39 (20.3) | 9 (15.8) | 0.567 |
| Central adenopathy | 134 (53.8) | 103 (53.6) | 31 (54.4) | 1 |
| Peripheral adenopathy | 96 (38.6) | 70 (36.5) | 26 (45.6) | 0.219 |
| Central nervous system | 6 (2.4) | 4 (2.1) | 2 (3.5) | 0.623 |
| Bone or joint | 36 (14.5) | 21 (10.9) | 15 (26.3) | 0.009 |
| Pericardial | 9 (3.6) | 6 (3.1) | 3 (5.3) | 0.433 |
| Abdominal | 47 (18.9) | 34 (17.7) | 13 (22.8) | 0.441 |
| Milary | 2 (0.8) | 1 (0.5) | 1 (1.8) | 0.406 |
| Other | 22 (8.8) | 17 (8.9) | 5 (8.8) | 1 |
| Microbiology or histology-confirmed diagnosis§ | 222 (89.2) | 167 (87.0) | 55 (96.5) | 0.051 |
| More than one site of TB disease§ | 96 (38.6) | 70 (36.5) | 26 (45.6) | 1 |
| Baseline steroid | 54 (21.7) | 42 (21.9) | 12 (21.1) | 1 |
| Other immunomodulatory drug¶ | 11 (4.4) | 10 (5.2) | 1 (1.8) | 0.465 |
| Adverse drug reaction recorded | 67 (26.9) | 52 (27.1) | 15 (26.3) | 0.864 |
| Hypercalcaemia before TB diagnosis | 15 (6.0) | 14 (7.3) | 1 (1.8) | 0.203 |
| Hypocalcaemia before TB diagnosis | 7 (2.8) | 4 (2.1) | 3 (5.3) | 0.362 |
| Calcaemia during anti-tuberculosis treatment | | | | |
| Hypercalcaemic | 10 (4.0) | 7 (3.6) | 3 (5.3) | |
| Hypocalcaemic | 4 (1.6) | 3 (1.6) | 1 (1.8) | |
| Normocalcaemic | 192 (77.1) | 144 (75.0) | 48 (84.2) | |
| Not recorded | 43 (17.3) | 38 (19.8) | 5 (8.8) | |

* All tests were Fisher’s exact test (categorical variables) or Wilcoxon rank-sum (numerical variables).
† Availability of baseline blood results: 222/249 had full blood; 209/249 had CRP; 226/249 had albumin; 113 had ESR; and 93/249 had vitamin D level available.
§ Diagnostic sample (e.g., fine-needle aspiration) was AFB-positive on microscopy, grew \( M. \) tuberculosis on culture or showed features consistent with TB disease on cytology or histology diagnosis.
¶ Included disease-modifying anti-rheumatic drugs and interferon therapy.
IQR = interquartile range; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; LDL = lower than the limit of detection; TB = tuberculosis; AFB = acid-fast bacilli.
Univariate associations with a paradoxical upgrading reaction

Variables associated with a PUR on univariate testing were lower age, the clinic site where treatment was given, lower lymphocyte count, having an AFB-positive diagnostic sample at baseline, having more than one site of TB disease at baseline and being prescribed vitamin D during anti-tuberculosis treatment (Table 2).

Multivariate associations with a paradoxical upgrading reaction

Although ethnicity was not significantly associated with a PUR (P = 0.374), it was considered to be a potential confounder because prescribing of vitamin D was influenced by patient ethnicity (Table 1, P = 0.039). The association between prescription of vitamin D and a PUR was adjusted for ethnicity in a logistic regression model, and remained significant, with an odds ratio (OR) of 3.35 (95%CI 1.59–7.06, P = 0.001; see Appendix http://rpubs.com/davidadambarr/EPTB-PUR-VitD). When the ethnicity variable was collapsed into three categories—white European, African, Asian (including Middle Eastern, South East Asian and South Asian) due to low frequencies in some of the pre-specified ethnic categories—prescription of vitamin D was associated with a higher rate of PUR in each grouping (Figure A).

The clinic site was also considered an important potential confounder because clinics had different rates of vitamin D prescribing and different rates of observed PURs. Clinic C was found to be an outlier with much lower rates of vitamin D prescribing and PUR than the other sites. Only 11/53 (21%) of patients at clinic C were prescribed vitamin D, and none had an observed PUR (Figure B). This ‘zero frequency cell’ in a contingency table of a PUR by clinic site and vitamin D prescription meant that clinic site could not be included in a full multivariate model. Instead, an exact logistic regression model was performed to adjust vitamin D prescription for clinic site. In this model, vitamin D prescription remained significant, with an OR of 3.70 (95%CI 1.54–13.23, P < 0.001; see Appendix http://rpubs.com/davidadambarr/EPTB-PUR-VitD). In addition, each patient prescribed vitamin D was matched with a control using a propensity score for vitamin D prescription based on all the variables associated with vitamin D prescription and a PUR (clinic site, age and ethnicity). In this analysis, patients prescribed vitamin

Table 2 Univariate associations with a PUR

|                      | No PUR (n = 212) | PUR (n = 37) | P value* |
|----------------------|-----------------|-------------|----------|
| Age, years, median [IQR] | 38.1 [30.0–52.8] | 30 [23.5–42.3] | 0.002† |
| Female               | 81 (38.2)       | 10 (27.0)    | 0.267    |
| Prescribed vitamin D | 40 (18.9)       | 17 (45.9)    | 0.001†   |
| Ethnicity            |                 |             | 0.374    |
| African              | 21 (9.9)        | 6 (16.2)     |          |
| Middle Eastern       | 2 (0.9)         | 0 (0.0)      |          |
| South East Asian     | 9 (4.2)         | 2 (5.4)      |          |
| South Asian          | 125 (59.0)      | 24 (64.9)    |          |
| White European       | 55 (25.9)       | 5 (13.5)     |          |
| Clinic site          |                 |             | 0.009*   |
| A                    | 92 (43.4)       | 12 (32.4)    |          |
| B                    | 49 (23.1)       | 13 (35.1)    |          |
| C                    | 50 (23.6)       | 3 (8.1)      |          |
| D                    | 21 (9.9)        | 9 (24.3)     |          |
| Baseline blood results, median [IQR] |          |             |          |
| Lymphocytes ×10³/l   | 1.43 [1.10–1.90] | 1.14 [0.83–1.51] | 0.013† |
| Monocytes ×10³/l     | 0.6 [0.47–0.80] | 0.66 [0.40–0.81] | 0.649    |
| Neutrophils ×10³/l   | 4.48 [3.40–5.93] | 4.95 [3.35–6.62] | 0.696    |
| Haemoglobin, g/l     | 130 [116–141]   | 124 [114–137] | 0.306    |
| ESR, mm/h            | 29 [13–49]      | 33 [10–61]   | 0.609    |
| Albumin, g/l         | 34 [30–38]      | 34 [29–38]   | 0.641    |
| CRP, mg/l            | 20 [5–55]       | 55 [13–73]   | 0.045†   |
| 25-hydroxycholecalciferol, nmol/l | 10 [LDL–20] | LDL [LDL–10] | 0.219    |
| More than one site of TB disease‡ | 76 (35.8) | 20 (54.1) | 0.044† |
| Diagnostic sample AFB-positive | 60 (28.3) | 20 (54.1) | 0.004† |
| Diagnostic sample culture-positive | 125 (59.0) | 28 (75.7) | 0.067    |
| Baseline steroid      | 42 (19.8)       | 12 (32.4)    | 0.128    |
| Other immunomodulatory drug§ | 11 (5.2) | 0 | 0.377    |

* All tests were Fisher’s exact test (categorical variables) or Wilcoxon rank-sum (numerical variables).
† Significant (P < 0.05).
‡ More than one of the following disease sites: pleural; central adenopathy; peripheral adenopathy; any intra-abdominal disease, pericardial, central nervous system, bone or joint, skin or eye. Miliary diagnosis automatically classified as > 1 site.
§ Included disease-modifying anti-rheumatic drugs and interferon therapy.

PUR = paradoxical upgrading reaction; IQR = interquartile range; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; LDL = lower than limit of detection; TB = tuberculosis; AFB = acid-fast bacilli.
D remained at greater risk for a PUR than propensity score-matched controls (OR 2.60, 95%CI 1.04–6.96, \( P = 0.046 \); see Appendix http://rpubs.com/davidadambarr/EPTB-PUR-VitD).

Finally, to create a multivariate model, all variables found to be significant on univariate testing except the clinic site were used. Ethnicity was also included as a potential confounder. In this ‘full’ model, younger age, an AFB-positive diagnostic sample, lymphocyte count and vitamin D prescription had largely unchanged OR estimates (Table 3). A more parsimonious model with variables deselected step-wise based on the Akaike Information Criterion was found to have equivalent fit and predictive performance. This model retained age, AFB status of the diagnostic sample, lymphocyte count, multiple sites of TB disease at baseline and vitamin D prescription as important independent variables (Table 3).

**DISCUSSION**

This is the first cohort study to examine the relationship between use of vitamin D and the risk of a PUR. We found a significant independent increased risk of a PUR to be associated with younger age, AFB positivity of the diagnostic sample, lymphopaenia, multiple sites of TB disease and receipt of vitamin D supplementation at baseline.

Younger age\(^4,18–20\) and lower lymphocyte count\(^5,19,21,22\) at the time of the TB diagnosis have been identified as risk factors for a PUR in previous cohort studies, and are mechanistically plausible mediators of the host immune response in a PUR. We also found having an AFB-positive diagnostic sample and multiple sites of TB disease at diagnosis to be risk factors for a PUR. Patients in this cohort were extensively investigated—92% underwent invasive sampling to attempt a microbiological or histological diagnosis before treatment—thereby reducing the risk of bias in these estimates. Several studies have found more extensive disease at baseline to be a risk factor,\(^3,18,22\) and a trend towards higher PUR in AFB-positive cases has also been described.\(^1\) More extensive disease and AFB-positive diagnostic sample variables suggest that a higher baseline antigen load is related to PUR development.

The active metabolite, 1\(\alpha\),25-dihydroxy vitamin D (1\(\alpha\),25(OH)2D3), supports an innate pro-inflammatory TLR-associated macrophage response in vitro, a response necessary for effective intracellular mycobacterial killing.\(^23\) Pre-treatment of monocytes with 1\(\alpha\),25(OH)2D3 induces cellular maturation and increased production of the innate cytokine tumour necrosis factor following lipopolysaccharide stimulation via TLR4 signalling.\(^24\) Mycobacterial stimulation of TLR1/2 on monocytes also leads to enhanced expression of the vitamin D receptor and 1\(\alpha\)-hydroxylase CYP27B1 and, in the presence of sufficient vitamin D, leads the antimicrobial activity via cathelicidin production.\(^23\) Enhancement of TLR signalling by supplementation with vitamin D in a patient with deficiency of vitamin D could therefore plausibly cause an upgraded innate immune response analogous to that seen in TB-IRIS.
Conversely, the effects of vitamin D on the adaptive immune system are thought to be anti-inflammatory, driving FoxP3 and CTLA4 expression, markers of regulatory T (Treg) cells and promoting type 2 T helper (Th2) cells, and blocking production of pro-inflammatory cytokines interleukin (IL) 2, IL-17 IL-21 and interferon-gamma. However, these observations vary according to the timing of treatment, the differentiation status of the vitamin D-treated cells and the presence of microbial products during treatment. Naïve CD4+ T-cells treated with active vitamin D suppress IL-4 production (the hallmark of Th2 cells), whereas co-treatment of CD4+ and CD8+ T cells with 1α,25(OH)2D3 and IL-4 induces IL-6 production.

Thus, vitamin D supplementation, depending on immune status and degree of antigen load, might just as much uncover or exacerbate pathological immune imbalances specific to some TB-susceptible hosts as it may prevent or resolve them. We speculate that supplementation of at-risk patients with high antigen load may lead to exacerbation of the innate TLR-mediated response, leading to exacerbated innate cytokine signalling similar to that observed in TB-IRIS. This response is also analogous to a reversal reaction in leprosy (progression from lepromatous to tuberculoid leprosy), and is associated with a switch in the inflammatory balance from phagocytic to vitamin D-mediated antimicrobial macrophage function and clearance of mycobacteria. A reversal reaction is also associated with increased FoxP3 staining in lesions, consistent with the role of vitamin D in Treg differentiation, as well as influx of Th1 cells.

The activation of the innate response by vitamin D primarily induces antimicrobial responses; a PUR could be associated with improved clearance of bacteria, but at the cost of increased inflammatory disease. This hypothesis suggests that the role of host-directed therapies (HDTs) such as vitamin D should be defined according to the specific clinical problem they are intended to solve, and that their effects may differ across the spectrum of TB disease and host response. Reassuringly, and as shown previously, a PUR was not associated with a high risk of serious adverse outcomes in our cohort, but did cause significant morbidity for some patients. Microbiological failure rates in EPTB cannot be routinely determined, but failures in clinical treatment or relapses in our low HIV, low multidrug-resistant TB setting were ~2%. To gain maximum utility from future HDTs, knowing which patients can benefit from enhanced bactericidal activity and which could benefit from anti-inflammatory therapy is necessary.

Weaknesses of this study stem from its retrospective design. The definition of a PUR had to be applied retrospectively due to differences between treatment clinicians in how formally these cases were diagnosed. Inter-rater agreement was, however, strong despite this limitation. The four clinic sites used in this study had markedly different patient characteristics. However, the clinic site could not be included in the final multivariate modelling due to the low frequency of events and vitamin D prescription at one clinic, this potential confounding could not be addressed fully. Finally, too few patients had serial serum levels of vitamin D checked to allow direct analyses of dose-concentration and PUR-response relationships, which would have provided a more robust test of a causal relationship between vitamin D and PURs, as would the measurement of vitamin D-associated inflammatory markers.

**CONCLUSIONS**

The PUR phenomenon is further evidence that, despite our current standardised approach to treatment, TB disease exists in a spectrum of host-
pathogen interactions. We should anticipate that the effects of future HDTs may be stratified by patient variables such as age, vitamin D status, lymphocyte count, antigenic load and inflammatory status. Most importantly, our results highlight that future trials of HDTs should consider adequate powering to detect PUR outcomes and prospectively define patient subgroups who may respond differently to these novel therapies.

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| Author, year, reference | Journal | Setting | Design | Total n | Site(s) of TB disease | Incidence Time from commenced treatment to onset of PUR | Risk factors | Risk factors |
|-------------------------|---------|---------|--------|---------|-----------------------|------------------------------------------------------|-------------|-------------|
| Ko, 2014 | Chest | Seoul, South Korea | Retrospective cohort | 315 | Pleural | 81/315 | Younger age, male sex, absence of comorbidities | Mean 76 days (range 16–233) |
| Kalita, 2014 | Int J Tuberc Lung Dis | Lucknow, India | Prospective cohort | 34 | CNS | 27/34 | NA | Majority seen on 3-month MRI scan |
| Park, 2013 | J Infect | Seoul, South Korea | Prospective cohort | 154 | Lymph node | 22/154 | Younger age | Median 4 months (IQR 2–9) |
| Olive, 2013 | Pedatr J Infect Dis | Brussels, Belgium | Retrospective cohort | 115 | Mixed | 12/115 | All participants were children, Younger age, absence of BCG, symptomatic at diagnosis | Median 39 days (range 15–75) |
| Geri, 2013 | Infection | Paris, France | Retrospective cohort | 76 | EPTB | 19/76 | Lymphopaenia, anaemia, peripheral adenopathy | Median 86 days (IQR, 36–125) |
| Jeon, 2012 | Int J Tuberc Lung Dis | Seoul, South Korea | Retrospective cohort | 458 | Pleural | 72/458 | Higher eosinophil count and lower protein in pleural fluid | Mean 8.8 weeks (SD 6.4) |
| Anuradha, 2011 | Int J Tuberc Lung Dis | Uttar Pradesh, India | Retrospective cohort | 110 | CNS | 7/110 | Younger age, ADRs, higher neutrophil count in pleural fluid at baseline, lower lymphocyte count in pleural fluid at baseline | NA |
| Jung, 2011 | Tohoku J Exp Med | Seoul, South Korea | Prospective cohort | 139 | Pleural | 32/139 | | Mean 51 days |
| Park, 2010 | J Infect | Seoul, South Korea | Prospective cohort | 75 | Lymph node | 8/75 had a post-therapy PUR; 1/75 had a PUR during therapy | NA | |
| Cho, 2009 | J Infect | Seoul, South Korea | Prospective cohort | 235 | Lymph node | 54/235 | Younger age, male, larger nodes at baseline, tender nodes at baseline, higher baseline neutrophils and monocytes | Median 8 weeks (IQR 4–14) |
| Cheng, 2007 | Int J Tuberc Lung Dis | Taipei, Taiwan | Retrospective cohort | 659 | Pulmonary | 16/659 | Anaemia, low BMI, hypoalbuminaemia, baseline lymphopaenia, change in lymph count at baseline to a PUR | Median 26 days (range 3–100) |
| Carvalho, 2006 | Clin Infect Dis | Brescia, Italy | Prospective cohort? | 137 | Mixed | 11/137 | Disseminated or EPTB at baseline | Median 107 days (range 31–443) |
| Hawkey, 2005 | Clin Infect Dis | Harrow, UK | Retrospective cohort | 109 | Lymph node | 25/109 | Higher monocyte count at baseline | Median 46 days (range 10–405) |
| Bøen, 2004 | Thorax | London, UK | Two retrospective cohorts, one HIV+ and other HIV− | 50+50 | Mixed | 5/50 HIV− | | Median 87 days (range 23–157) |
| Cheng, 2003 | Eur J Clin Microbiol Infect Dis | Hong Kong | Prospective cohort | 104 | Mixed | 16/104 | EPTB at baseline; lymphopaenia at baseline; change in lymph count baseline to PUR | Median 56 days (range 20–109) |
| Choi, 2002 | Radiology | Seoul, South Korea | Retrospective cohort | 141 | Pleural | 16/141 | patients developed new CXR lesions | Mean 3 months (range 1–9) |
Table A.1 (continued)

| Author, year, reference | Journal | Setting | Design | Total n | Site(s) of TB disease | Incidence | Risk factors | Time from commenced treatment to onset of PUR |
|-------------------------|---------|---------|--------|---------|-----------------------|-----------|-------------|-----------------------------------------------|
| Memish, 200017          | Clin Microbiol Infect | Riyadh, Saudi Arabia | Retrospective case series | 99 | Lymph node | 6/99 worsening, but relapse not excluded | NA | NA |
| Al-Majed, 199618        | Respir Med | Riyadh, Saudi Arabia | Retrospective cohort | 61 | Pleural | 10/61 NA | NA | Median 3 weeks (range 1–4) |

TB = tuberculosis; PUR = paradoxical upgrading reaction; CNS = central nervous system; NA = not available; BCG = bacille Calmette–Guerin; EPTB = extra-pulmonary TB; IQR = interquartile range; SD = standard deviation; ADR = adverse drug reaction; BMI = body mass index; HIV = human immunodeficiency virus; + = positive; − = negative; CXR = chest X-ray.
Table A.2 Definitions of variables in raw data

| Variable | Definition |
|----------|------------|
| DAB | Index for a PUR diagnosis by reviewer DAB; PUR = probable or definite PUR. No_PUR = unlikely or no PUR |
| RAS | Index for a PUR diagnosis by reviewer RAS; PUR = probable or definite PUR. No_PUR = unlikely or no PUR |
| DJB | Index for a PUR diagnosis by reviewer DJB; PUR = probable or definite PUR. No_PUR = unlikely or no PUR |
| PUR | PUR = minimum 2/3 reviewers classified as a PUR. No_PUR = maximum one reviewer classified as a PUR |
| PUR.date | Date of a PUR diagnosis (not date of symptom onset) |
| PUR.Rx | Any treatment prescribed for PUR management |
| Ethnicity | As recorded in clinical notes, and classified by study authors into five categories: South Asian, White European, sub-Saharan African, South-East Asian, West Asian (Middle East, including North African and Eastern Mediterranean). In some cases where ethnicity was not recorded in the clinical notes, but country of origin for first-generation immigrants to Scotland was, the latter was used as a proxy |
| Clinic | Patients included in the cohort were recruited from four Glasgow centres treating TB cases, designated A, B, C, and D here. All are tertiary hospitals providing in-patient and out-patient TB care |
| Pleural | Yes/no: pleural disease diagnosed by clinicians responsible for patient’s care at the time of the TB diagnosis |
| Internal.LN | Yes/no: intra-thoracic and/or intra-abdominal lymph node disease identified at the time of the TB diagnosis on imaging studies |
| External.LN | Yes/no: extra-thoracic/extra-abdominal (i.e., cervical, axillary, etc.) lymph node disease diagnosed by clinicians responsible for patient’s care at the time of the TB diagnosis on clinical examination or imaging studies, ± histological or microbiological evidence |
| CNS | Yes/no: disease in the central nervous system diagnosed by clinicians responsible for patient’s care at the time of the TB diagnosis |
| BJI | Yes/no: bone or joint disease diagnosed by clinicians responsible for patient’s care at the time of the TB diagnosis |
| Pericardial | Yes/no: pericardial disease diagnosed by clinicians responsible for patient’s care at the time of the TB diagnosis |
| Abdominal | Yes/no: intra-abdominal disease (including genito-urinary, liver, spleen, gastro-intestinal tract sites) diagnosed by clinicians responsible for patient’s care at the time of the TB diagnosis |
| Military | Yes/no: miliary disease diagnosed by clinicians responsible for patient’s care at the time of the TB diagnosis |
| Other.site | Yes/no: any other site of disease diagnosed (including skin, eyes) by clinicians responsible for patient’s care at the time of the TB diagnosis |
| Microscopy.diagnosis | positive/negative: acid-fast bacilli identified in any clinical sample taken pre-treatment |
| Culture.diagnosis | positive/negative: a clinical sample taken pre-treatment that grew M. tuberculosis upon culture |
| Histology.diagnosis | positive/negative: a biopsy or cytology sample showed evidence of TB infection (including any granulomatous inflammation) |
| Basis.diagnosis | ‘micro_or_histo_confirmed’ if positive microscopy, culture or histology as defined above; otherwise ‘clinical_diagnosis’ |
| Baseline.steroid | Yes/no: patient was started on corticosteroid therapy at the same time as anti-tuberculosis treatment was initiated |
| Other.immuno.drug | Yes/no: patient taking any of the following during anti-tuberculosis treatment: oncological chemotherapy, interferon therapy, anti-inflammatory DMARDs or any monoclonal antibody preparation |
| Lymphocytes | Lymphocyte count at baseline (≤ 1 week of TB treatment start date), 10⁹/l |
| Neutrophils | Neutrophil count at baseline (≤ 1 week of TB treatment start date), 10⁹/l |
| Haemoglobin | Haemoglobin concentration at baseline (≤ 1 week of TB treatment start date), g/dl |
| ESR | ESR at baseline (≤ 2 week of TB treatment start date), mm/h |
| Albumin | Albumin count at baseline (≤ 1 week of TB treatment start date), g/l |
| CRP | CRP concentration at baseline (≤ 1 week of TB treatment start date), mg/l |
| Prior.hypercalcaemia | Any adjusted calcium level greater than local reference range recorded in the 3 months before starting anti-tuberculosis treatment |
| Prior.hypocalcaemia | Any adjusted calcium level less than local reference range recorded in the 3 months before starting anti-tuberculosis treatment |
| Calcaemia.during.TBRx | Any adjusted calcium level greater than local reference range during treatment = hypercalcaemia; any adjusted calcium level less than local reference range during anti-tuberculosis treatment = hypocalcaemia; if blood calcium was not checked during anti-tuberculosis treatment = NA |
| VitD.Rx.Date | Date of first vitamin D prescription during anti-tuberculosis treatment |
| Date.TB.Rx.start | Date of first dose of anti-tuberculosis chemotherapy |
| Date.TB.Rx.finish | Date of first dose of anti-tuberculosis chemotherapy |
| VitD.Rx.Date | Date of first vitamin D prescription during anti-tuberculosis treatment |
| age | Age at the TB diagnosis |

PUR = paradoxical upgrading reaction; TB = tuberculosis; DMARD = disease-modifying anti-rheumatic drug; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; NA = not available; IU = international unit; ADR = adverse drug reaction.
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OBJECTIFS : Les réactions paradoxales à la tuberculose (TB) sont un exemple notable de notre compréhension incomplète de l’interaction hôte pathogène pendant le traitement de la TB. Nous avons voulu déterminer les facteurs de risque de réaction paradoxale à la TB et spécifiquement évaluer une association indépendante avec la prescription de vitamine D.

SCHEMA : Des patients adultes consécutifs négatifs à l’infection pour le virus de l’immunodéficience humaine (VIH) traités pour une TB extrapulmonaire ont été identifiés à partir d’une base de données de Surveillance Etendue des Infections à Mycobactéries. Dans notre contexte, la vitamine D est prescrite de manière variable aux patients TB nouvellement diagnostiqués. Une définition précédemment publiée des réactions paradoxales à la TB a été rétrospectivement appliquée aux dossiers électroniques centralisés des patients et les données de tous les facteurs de risque précédemment décrits ont été extraites de ces dossiers. L’association de la prescription de vitamine D a été évaluée par régression logistique multivariable.

RESULTATS : La majorité des 249 patients inclus avait des adénopathies TB ; 222/249 avaient une TB confirmée par microbiologie et/ou par histologie. La vitamine D a été prescrite à 57/249 (23%) patients ; 37/249 (15%) ont été classés comme ayant des réactions paradoxales. Les facteurs de risque indépendants trouvés ont été le jeune âge, un échantillon positif aux bacilles acido-alcoolo-résistants, de multiples sites de maladie, une numération de lymphocytes plus faible et la prescription de vitamine D.

CONCLUSION : Nous spéculons que les cellules immunitaires pro-inflammatoires innées médiane par la vitamine D, avec une charge antigénique élevée, pourraient être à l’origine des réactions paradoxales pendant le traitement de la TB.