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# Vitamin D deficiency is associated with tuberculosis disease in British children

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## Title (complete)

Vitamin D deficiency is associated with tuberculosis disease in British children

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Vitamin D deficiency and tuberculosis disease


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## Conflicts of interest

None declared

## Author contributions

BK conceived and led the NIKS study. DK managed study recruitment and data. JB, JP, AW, BW and PM recruited patients into the study. BW and AJM conceived and undertook this analysis and drafted the manuscript. All authors contributed to the final manuscript.

## SUMMARY

### Background

Basic science, epidemiological and interventional research supports a link between vitamin D and tuberculosis immunity, infection and disease. We evaluated the association between vitamin D levels and tuberculosis (TB) infection and disease in UK children recruited to the NIHR IGRA Kids Study (NIKS).

### Methods

Children presenting between 2011-2014 were eligible if they had history of exposure to an adult case with sputum smear/culture-positive TB, or were referred and diagnosed with TB disease. Children were assessed at baseline and 6-8 weeks for immunological evidence of TB infection (IGRA and/or tuberculin skin test) and evidence of TB disease. Some centres routinely measured total 25-hydroxy vitamin D levels.

### Results

166 children were included. Median 25-hydroxy vitamin D levels were higher in uninfected children (45.5 nmol/l) compared to those with infection (36.2 nmol/l) and disease (20.0 nmol/l). The difference between TB infection and disease was statistically significant ($p<0.001$). By logistic regression, lower vitamin D levels were associated with TB disease among participants with infection/disease, with no evidence of confounding by age, sex, BCG status, ethnicity, non-contact referral, season or centre.

### Conclusion

Children with TB disease had lower vitamin D levels than children with infection. Implications for prevention and treatment remain to be established.

*Keywords*
Tuberculosis
Children
Vitamin D

## *Main body*

Tuberculosis (TB) is an important cause of childhood morbidity and mortality; the World Health Organization (WHO) estimated that in 2017 one million children globally fell ill with TB disease and 234,000 children died.[1,2] Following exposure to infectious TB, about half of children will develop TB infection,[3] defined as an absence of symptoms and evidence of immunological sensitisation to *M. tuberculosis*. The two most commonly used immunological tests to identify TB infection are the tuberculin skin test (TST) and interferon gamma release assay (IGRA). Both have limitations.[4–6] Of those who become infected, most will contain the initial infection and remain well; a minority will progress to TB disease soon after the initial exposure or following a period of infection.

The reasons why outcomes differ for children despite similar exposures are incompletely understood, but amongst host factors young age and immunosuppression are associated with progression to TB disease.[7–9] Epidemiological evidence shows association between vitamin D level and TB infection and disease, but its role is incompletely understood.[10–17] Vitamin D has been shown to upregulate the expression of the anti-microbial peptide cathelicidin, which has anti-mycobacterial activity.[18]

The relationship between vitamin D status and TB disease was first reported in 1985 where adults with untreated TB disease were found to have lower levels of vitamin D than matched controls.[16] This finding has been replicated across multiple adult case-control studies, as summarised in a 2007 meta-analysis.[13]

Children attending TB clinics with a diagnosis of either TB infection or TB disease are frequently found to be vitamin D deficient or insufficient.[14] We aimed to investigate the relationship between vitamin D status, measured as 25-hydroxyvitamin D (25-OHD), and risk of TB disease or TB infection in children presenting with TB disease and those proactively assessed following close exposure to an adult with smear or culture-positive pulmonary TB.

## METHODS

### Study Setting

The **N**IHR-funded **I**GRA **K**ids **S**tudy (NIKS; UKCRN 10069) aimed to measure incident TB disease in children found to be TST positive and IGRA negative following close exposure to an infectious TB case,[19] in order to evaluate a revision to the National Institute for Health and Care Excellence (NICE) screening guideline in the United Kingdom (UK).[20] Children aged under 17 years presenting to one of the 11 participating centres between 1st Jan 2011 and 31st Dec 2014 were eligible for recruitment if they had a history of exposure to an index case with sputum smear-positive or culture-positive TB. The NIKS study was powered to measure incident TB disease in TST-positive but IGRA-negative children. We also recruited children who presented with TB disease. Recruitment has been described in detail elsewhere.[19,21] Notably, prior publications only include children referred on the basis of an infectious *household* contact. Five of the 11 centres measured 25-OHD levels in a proportion of children. These data provide a convenience sample for this vitamin D substudy.

### Study procedures and definitions

Following the diagnosis of an adult with sputum smear-positive or culture-positive pulmonary TB, household contacts were identified and assessed for evidence of TB infection and TB disease by local TB teams as per their usual clinical practice. Screening for TB infection involved both TST and IGRA. TST was performed by trained clinicians by intradermal injection of two tuberculin units (purified protein derivative RT23, Statens Serum Institute). The results were read at 48-72 hours. The type of IGRA test used was dependent on the usual practice of the participating clinic: either QuantiFERON-TB Gold In-Tube (Cellestis Ltd; in 4 centres) or T-SPOT.TB (Oxford Immunotec Ltd; in 1 centre). Testing and interpretation of the results followed the manufacturer's specifications. Children with initial negative or indeterminate IGRA or TST underwent repeat testing after 6-8 weeks. In addition, symptomatic children were recruited if a decision to treat for TB disease was made by the responsible clinician.

Investigation for TB disease in children included a history of suggestive symptoms, clinical examination, TST, IGRA and chest radiography. Microbiological confirmation was sought wherever feasible. 25-OHD levels in blood were requested as per usual practice of the treating clinician and evaluated in individual hospital laboratories, which all participate in the DEQAS scheme to ensure the the analytic reliability of 25-OHD. Written consent was obtained for recruitment into the study.

### Case definitions

Immunological evidence of TB infection was defined as: (a) a positive IGRA test or (b) TST of 15 mm or greater if BCG vaccinated or (c) TST of 6mm or greater if BCG unvaccinated as per NICE guidance in 2011.[20]

TB infection was defined as immunological evidence of TB in an asymptomatic patient with normal chest radiography. As per the WHO case definition,[2] TB disease was defined as at least one sign or symptom of TB or abnormal chest radiography and a decision to treat for TB disease by the responsible clinician, or bacteriological proof of TB disease through culture or PCR, combined with characteristic symptoms and signs. The child was considered to be vitamin D deficient if the 25-OHD level was less than 25 nmol/L, insufficient when the level was between 25 and 50 nmol/L and sufficient when the level was greater than 50 nmol/L as per European Society of Paediatric Gastroenterology Hepatology and Nutrition Guidelines.[22,23]

Three groups of children were therefore identified: 1) uninfected child contacts (*uninfected* group); 2) child contacts with TB infection (*TB infection* group); 3) children diagnosed with TB disease identified either through contact tracing or passively presenting with symptoms and signs (*TB disease* group).

### Statistical analysis

A bespoke database was used for real-time data-entry and later checked centrally for entry errors. Data were analysed using Microsoft Excel 2010 (Seattle, WA) and R for Windows 3.2.1 (R Foundation

for Statistical Computing, Vienna). Differences in 25-OHD levels were assessed between groups of patients using two-sided t-tests on square-root transformed data to account for negative skew. The relationship between square-root 25-OHD levels and clinical states was assessed using logistic regression. Confounding by sex, age, BCG status, ethnicity, reason for referral, season and site was tested for by adding these to the models individually, and a multivariable model produced by inclusion of all significant predictors ($p \leq 0.05$) and stepwise removal of least significant predictors.

### *Ethics*

The study was approved by the UK National Research Ethics Service (Rec: 11/11/11)

## RESULTS

Of 513 children originally recruited into the NIKS study, 187 presented to the 5 study sites included here. 25-OHD results were available for 168 children (90%). Children without vitamin D results were younger (p=0.04 by Wilcoxon rank sum), but no significant differences were noted in gender, ethnicity, BCG vaccination, reason for referral or prevalence of testing for HIV. Prevalence of TB disease was higher in centres testing vitamin D (15% vs. 6%, see supplementary table 1).

Two children were excluded (one indeterminate IGRA and one IGRA missing). Of 35 children in the *TB disease* group, 26 were diagnosed with TB disease at initial evaluation following referral due to TB exposure and 8 were external referrals because of symptoms and signs of TB disease. One child was diagnosed with TB infection at baseline but at the 6-8 week follow-up was diagnosed with TB disease and was included in the *TB disease* group. Of 57 children in the *TB infection* group, 49 were diagnosed at baseline and 8 developed evidence of infection at follow-up. Seventy-four children were classed as *uninfected*. Those diagnosed with TB disease were older and more likely to be of black ethnicity. Demographic characteristics and results are shown in table 1.

Two uninfected children with outlying 25-OHD results (>100 nmol/l above the next highest result) were excluded, since they could inflate an apparent association and are most likely to represent

recent high-dose supplementation. There was no evidence of association between 25-OHD and season (p=0.635 by ANOVA). Vitamin D levels differed by centre (p<0.001 by ANOVA) with lower median in St Mary's Hospital and Manchester sites (18.5 and 18.2 nmol/l respectively), intermediate median in Northwick Park Hospital (31 nmol/l) and higher medians in Bristol Royal Infirmary and Glasgow Yorkhill Hospital (45 and 51.5 nmol/l respectively).

*Uninfected* children had a median 25-OHD level of 46 nmol/l (IQR 25-65); children with *TB infection* had a median level of 36 nmol/l (IQR 23-60); children in the *TB disease* group had a median of 20 nmol/l (IQR 12.5-35.5; Table 1). Children with TB disease had significantly lower 25-OHD levels than those with TB infection (p<0.001; see figure 2). The difference between uninfected children and those with TB infection was not statistically significant (p=0.63). Since only 8 children progressed to develop evidence of TB infection and only one progressed from TB infection to TB disease, meaningful comparison is precluded.

In a logistic regression analysis, lower 25-OHD levels were associated with TB disease among patients with TB infection or disease, with no evidence of confounding by age, sex, BCG status, ethnicity, referral for symptoms, season or centre individually or in a multivariable model (table 2 and supplementary table 2). The unadjusted odds ratio of 0.65 (95% confidence interval 0.51-0.85). corresponds to a predicted probability of TB disease among children with TB infection and disease of 62% for the 25$^{th}$ centile 25-OHD level (20 nmol/l) and 30% for the 75$^{th}$ centile (60 nmol/l). Restricting the analysis to the three centres with TB disease cases did not materially change the odds ratio (0.66 [0.50-0.87]).

## DISCUSSION

In the context of a household TB contact study, we describe the relationship between TB classification and vitamin D status in a cohort of 168 British children. Consistent with other reports, we show a stepwise decline in 25-OHD levels from uninfected children to those with TB infection and

then children with TB disease, with a significant difference between those with TB infection and TB disease.

An Australian retrospective study showed an association between vitamin D deficiency (<25 nmol/l 25-OHD) and both TB disease and TB infection in adult sub-Saharan African immigrants attending infectious diseases and refugee health clinics.[11] A small study from a refugee clinic in Australia found that children with TB disease and TB infection had lower 25-OHD levels than uninfected children.[12]

A larger study with children from both Italy and the UK found a higher risk of vitamin D deficiency (<25 nmol/l 25-OHD) both in those with TB disease and with TB infection.[15] While efforts were made in this study to account for confounding factors, most of the children with TB disease were recruited from UK hospitals, whereas the control and infected children were largely recruited from Italy where sunlight-dependent vitamin D production is greater. Further, the children were a mixed population comprising healthy recent immigrants with no known exposure to TB, children with clinical suspicion of TB and contacts of cases, raising the possibility of spectrum bias. In a further study in Pakistan, the risk of progression to TB disease was investigated in a cohort of 109 household contacts of sputum smear-positive source cases, of whom 44 were children under the age of 18.[17] A 1-log fall in 25-OHD level was associated with a five-fold increased risk (95% confidence interval 1.2-21.3) of progression to TB over two years. Most recently, a study of nearly 10,000 Mongolian schoolchildren found vitamin D deficiency (defined as <10 ng/ml [~25 nmol/l] 25-OHD) was associated with a 23% (95% confidence interval 8-40%) increased risk of QuantiFERON positivity (maintained when those with TB disease were excluded).[24]

The findings from these studies are similar to ours, demonstrating an association between lower vitamin D levels in children with TB infection and disease. However, these studies lack the larger numbers of children we present, and the children in our study were recruited in the context of household exposure to infectious TB.

In our study we demonstrate an association between lower vitamin D levels and TB disease among patients with TB infection or disease. The association is strong with no evidence of confounding by demographic factors, BCG vaccination, reason for referral, season or site. A causal association would be very important, since vitamin D deficiency is common and easily treatable.

Our study benefits from being large and comprising a representative population of children most of whom were referred on the basis of recent household contact history alone, thus avoiding spectrum bias. We used clear diagnostic definitions, standardised assessment for TB infection with both TST and IGRA and considered potential confounders. As with all research in this area, we are limited in our ability to diagnose TB infection. The proportion of children undergoing vitamin D measurement in the 5 centres was high, and the prevalence of TB was higher in contacts referred to these centres, increasing the power to detect a difference.

Being a cross-sectional study, we can draw limited conclusions regarding causality. It is possible that low vitamin D levels predispose to progression from infection to disease. However, it is also conceivable that being infected or having TB disease reduces vitamin D levels. There is also the possibility of residual confounding by unmeasured factors associated with vitamin D levels which might influence the risk of progressing to infection or disease.[25] The lack of an apparent association with TB infection and disease overall may represent a type 2 error, but it is conceivable that vitamin D is only associated with progression from infection to disease.

*In vitro*, it has been demonstrated that a single dose of vitamin D enhances immunity to *Mycobacteria.*[26] However, there are inconsistent reports of the effects of vitamin D supplementation in the treatment of TB with some studies reporting beneficial effects[27,28] and others, no effect.[29] A recent individual patient data meta-analysis found evidence of accelerated sputum smear conversion only in children with multi-drug-resistant TB (disease caused by organisms resistant to isoniazid and rifampicin).[30]

Vitamin D deficiency is known to be prevalent in children attending TB clinics, a finding confirmed in the present study. Since it is recommended to treat vitamin D deficiency in children to prevent other problems[31] we propose that vitamin D levels should be routinely tested in those exposed to TB and supplements given to those with sub-optimal levels. We provide additional evidence to support the epidemiological association between lower vitamin D levels and TB disease. Further cohort studies would need to consider confounding carefully. To explore a causal hypothesis, children exposed to an infectious pulmonary TB source case could be followed longitudinally to determine if 25-OHD levels at baseline predict which children will develop TB infection and TB disease. 25-OHD levels could also be compared between siblings discordant for TB status. The role of vitamin D supplementation for all children exposed to infectious TB source cases also requires further investigation.

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## TABLES

Table 1: Demographic and clinical characteristics of children in the study.

| | | Uninfected | TB infection | TB disease |
|---|---|---|---|---|
| Number included in current study | | 74 | 57 | 35 |
| Male | | 37 (50%) | 29 (51%) | 20 (57%) |
| Median age (years) and IQR | | 3.8 (1.5-7.4) | 6.6 (3.1-9.6) | 9.6 (4.5-12.5) |
| Ethnicity | Black | 23 (31%) | 20 (35%) | 20 (57%) |
| | White | 22 (30%) | 25 (44%) | 4 (11%) |
| | Asian | 25 (34%) | 8 (14%) | 8 (23%) |
| | Mixed/Other | 4 (5%) | 4 (7%) | 3 (9%) |
| HIV tested* | | 53 (72%) | 44 (77%) | 30 (86%) |
| BCG: documentation or scar | | 48 (65%) | 34 (60%) | 24 (69%) |
| Site | Northwick Park | 25 | 17 | 10 |
| | St Mary's | 7 | 3 | 10 |
| | Yorkhill | 21 | 16 | 0 |
| | Bristol Royal | 21 | 13 | 5 |
| | Manchester sites | 0 | 8 | 0 |
| 25-OHD level | nmol/l (IQR) | 45.5 (25.0-65.0) | 36.2 (23.0-60.0) | 20.0 (12.5-33.5) |

| | | | | |
|---|---|---|---|---|
| | Sufficient/Optimal | 33 (45%) | 17 (30%) | 4 (11%) |
| | Insufficient | 23 (31%) | 25 (44%) | 9 (26%) |
| | Deficient | 18 (24%) | 15 (26%) | 22 (63%) |

TB: tuberculosis; BCG: Bacillus Calmette–Guérin; IQR: inter-quartile range; 25-OHD: 25-hydroxyvitamin D

*No children were found to be HIV positive.

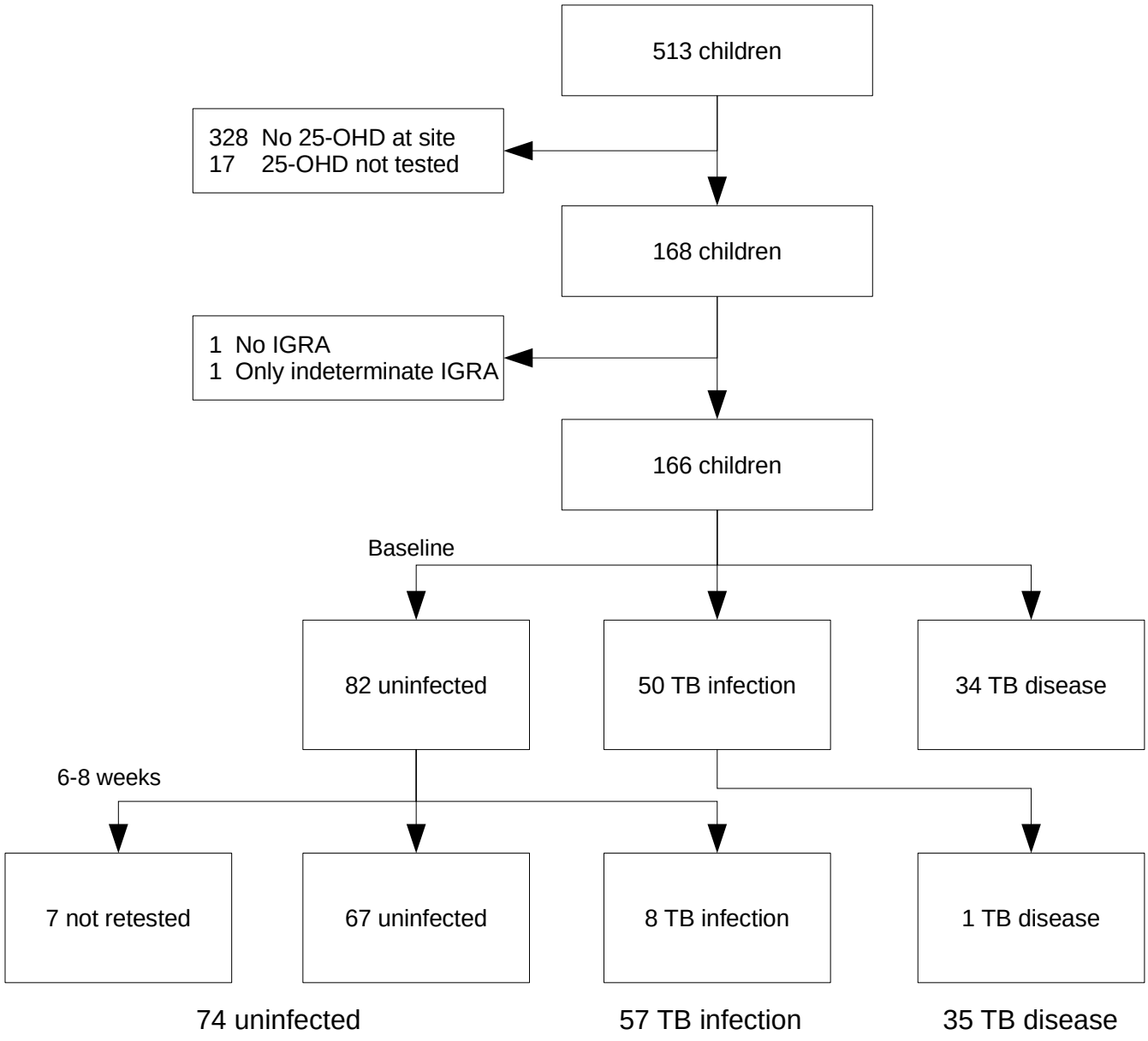
Table 2: Univariable odds ratios for 25-OHD and potential confounders as predictors of TB disease among those with TB infection or TB disease. Multivariable odds ratios presented for predictors remaining by stepwise removal. CI: confidence interval; OR: odds ratio; 25-OHD: 25-hydroxyvitamin D. *No TB cases

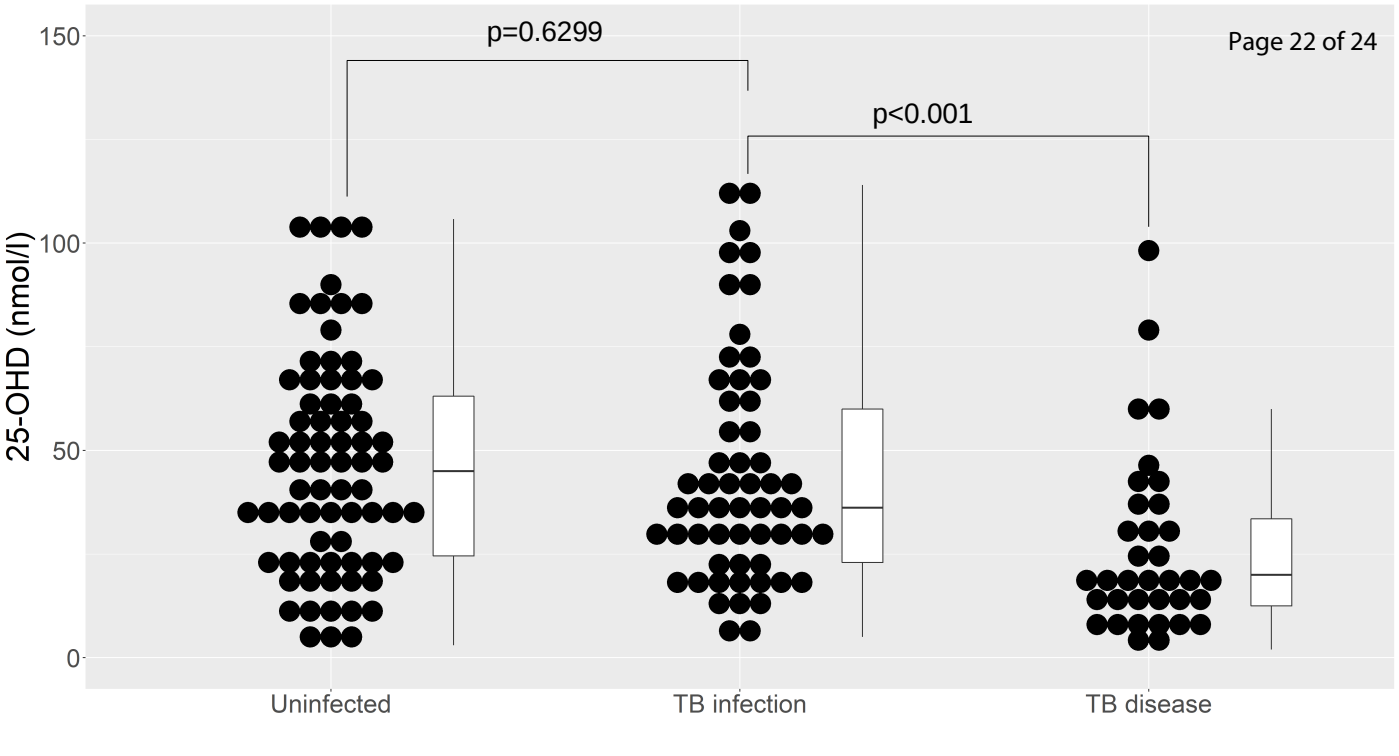
| | Univariable OR (95% CI) | P value | Multivariable OR | Multivariable p value |
|---|---|---|---|---|
| **Square root 25-OHD** (nmol/l$^{0.5}$) | 0.65 (0.51-0.85) | 0.001 | 0.67 (0.51-0.87) | 0.002 |
| **Male** | 1.29 (0.55-3.00) | 0.56 | - | - |
| **BCG** | 1.48 (0.61-3.59) | 0.39 | - | - |
| **Black ethnicity** | 2.47 (1.04-5.84) | 0.04 | - | - |
| **Age** (years) | 1.11 (1.01-1.23) | 0.03 | - | - |
| **Referred for symptoms** | 16.6 (1.97-139) | 0.01 | 15.3 (1.6-147.4) | 0.02 |
| **Season** (baseline Autumn) | - | - | - | - |
| **Spring** | 4.50 (1.40-14.5) | 0.01 | | |
| **Summer** | 1.20 (0.30-4.79) | 0.8 | | |
| **Winter** | 2.50 (0.81-7.73) | 0.11 | | |
| **Centre** (baseline Bristol) | - | - | - | - |
| **Glasgow Yorkhill** | N/A* | - | | |
| **St Mary's** | 8.67 (1.66-45.2) | 0.01 | | |
| **Northwick Park** | 3.06 (0.91-10.3) | 0.07 | | |
| **Manchester sites** | N/A* | - | | |

## FIGURE LEGENDS

**Figure 1**: Flow diagram for inclusion into the study, with outcomes for initial and repeat testing.

**Figure 2**: 25-OHD by clinical category with p-values for differences of square-root transformed values by t test. Two uninfected children with outlying 25-OHD results were excluded (>100 nmol/l above the next highest result) likely representing recent high-dose supplementation. Box shows median and interquartile range (IQR). Whiskers extend to cover the samples no further than 1.5 times with IQR away from 25$^{th}$ and 75$^{th}$ percentiles. 25-OHD: 25-hydroxyvitamin D


513 children
328 No 25-OHD at site
17 25-OHD not tested
168 children
1 No IGRA
1 Only indeterminate IGRA
166 children
Baseline
82 uninfected
50 TB infection
34 TB disease
6-8 weeks
7 not retested
67 uninfected
8 TB infection
1 TB disease
74 uninfected
57 TB infection
35 TB disease


p=0.6299
p<0.001
150
100
50
0
25-OHD (nmol/l)
Uninfected
TB infection
TB disease

| | Centres measuring 25-OHD | | | Other centre |
|---|---|---|---|---|
| | 25-OHD available | 25-OHD unavailable | P value | |
| **Number** | 168 | 19 | - | 326 |
| **Referred due to symptoms** | 8 (5%) | 1 (5%) | 1 | 4 (1%) |
| **Contact outcomes**<br>TB disease<br>TB infection<br>No TB | <br>35 (21%)<br>57 (34%)<br>76 (45%) | <br>1 (5%)<br>6 (32%)<br>11 (58%) | <br><br>0.52 | <br>21 (6%)<br>106 (33%)<br>195 (60%) |
| **Male** | 86 (49%) | 11 (58%) | 0.63 | 163 (50%) |
| **Median age** (years) with interquartile range | 5.6 (2.5-10.7) | 2.7 (1.4-6.7) | 0.04 | 6.5 (3.2-11.4) |
| **Ethnicity**<br>Black<br>White<br>Asian<br>Mixed/Other/Unknown | <br>63 (38%)<br>51 (30%)<br>43 (26%)<br>11 (7%) | <br>8 (42%)<br>5 (26%)<br>6 (32%)<br>0 | <br><br>0.80 | <br>66 (20%)<br>70 (21%)<br>149 (46%)<br>41 (13%) |
| **HIV tested*** | 129 (77%) | 12 (63%) | 0.26 | 315 (97%) |
| **BCG**: documentation or scar | 108 (64%) | 12 (63%) | 1 | 241 (74%) |

Supplementary table 1: clinical and demographic variables for children stratified by centre (measuring 25-OHD or not) and availability of 25-OHD result. P values compare those with and without 25-OHD results within centres that measured 25OHD levels. Fisher's exact test used for proportions, one-way ANOVA used for categorical variables, and Wilcoxon rank sum for continuous data. *All HIV tests were negative. 25-OHD: 25-hydroxyvitamin D; ANOVA: analysis of variance; HIV: Human Immunodeficiency Virus; TB: tuberculosis.

| | Odds ratio (95% CI) | 25-OHD p value |
|---|---|---|
| **Unadjusted** | 0.65 (0.51-0.85) | 0.001 |
| **Male** | 0.65 (0.50-0.84) | 0.001 |
| **BCG** | 0.65 (0.50-0.85) | 0.001 |
| **Black ethnicity** | 0.67 (0.51-0.87) | 0.003 |
| **Age** (years) | 0.68 (0.52-0.88) | 0.004 |
| **Referred for symptoms** | 0.68 (0.52-0.88) | 0.004 |
| **Season** | 0.68 (0.52-0.88) | 0.004 |
| **Centre** | 0.72 (0.53-0.96) | 0.03 |

Supplementary table 2: Odds ratio and p values for 25-OHD as a predictor of TB disease among those with TB disease and TB infection (n=92) unadjusted and adjusted for single potential confounders. 25OHD: 25-hydroxyvitamin D; TB: tuberculosis; BCG: bacille calmette-guerin