ORIGINAL ARTICLE

Vitamin D deficiency and TB disease phenotype

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▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/thoraxjnl-2014-206617).

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Received 1 December 2014
Revised 6 March 2015
Accepted 14 April 2015
Published Online First
23 September 2015

ABSTRACT

Background Extrapulmonary TB is increasingly common, yet the determinants of the wide clinical spectrum of TB are poorly understood.

Methods We examined surveillance data (Birmingham, UK: 1980–2009 and USA Centers for Disease Control: 1993–2008) to identify demographic factors associated with extrapulmonary TB. We then directly tested association of these factors and serum 25-hydroxycholecalciferol (25(OH)D) concentration with extrapulmonary TB by multivariable analysis in a separate UK cohort.

Results Data were available for 10 152 and 277 013 TB cases for Birmingham and US, respectively. Local-born individuals of white ethnicity had a lower proportion of extrapulmonary disease when compared with local-born non-whites (p<0.0001); both groups had a lower proportion of extrapulmonary disease when compared with foreign-born non-whites (p<0.0001). In a separate UK cohort (n=462), individuals with extrapulmonary TB had lower mean serum 25(OH)D concentration than those with pulmonary TB (11.4 vs 15.2 nmol/L, respectively, p=0.0001). On multivariable analysis, vitamin D deficiency was strongly associated with extrapulmonary TB independently of ethnicity, gender and other factors. Doubling in serum 25(OH)D concentration conferred substantially reduced risk of extrapulmonary disease (OR 0.55, 95% CI 0.41 to 0.73).

Conclusions We identify vitamin D deficiency as a probable risk factor for extrapulmonary dissemination in TB, which may account for the associations of dark-skinned ethnicity and female gender with extrapulmonary disease. Our findings implicate vitamin D status in *Mycobacterium tuberculosis* containment in vivo and, given the high prevalence of deficiency, may inform development of novel TB prevention strategies.

Key messages

What is the key question?
- What are the factors that result in extrapulmonary TB phenotype?

What is the bottom line?
- Vitamin D deficiency is strongly and independently associated with extrapulmonary TB at the time of TB diagnosis.

Why read on?
- Our data provide the first evidence for vitamin D determining TB disease phenotype and help to explain the longstanding yet unexplained association of extrapulmonary TB with ethnicity and geographical origin.

INTRODUCTION

Although TB presents as a broad spectrum of clinical phenotypes, clinically categorised as pulmonary and extrapulmonary disease, little is known about the determinants of this clinical spectrum. Although pulmonary TB is the commonest presentation, extrapulmonary TB accounts for a large and growing proportion.[1 2]

The most important known risk factors for extrapulmonary TB are untreated HIV infection, iatrogenic immunosuppression (especially anti-tumour necrosis factor-α therapy) and infancy.[3 4] This association with impaired cellular immunity suggests that extrapulmonary disease in immunocompetent adults may reflect a more subtle impairment of host responses that mediate bacterial containment in vivo. Genetic factors associated with extrapulmonary TB include polymorphisms in vitamin D receptor,[5 6] interleukin-1β,[6 7] Toll-like receptor 2 (TLR2/1)[6] and the P2X7 gene, suggesting a role for innate immunity in containment versus extrapulmonary dissemination.[8] Pathogen-related factors may also influence disease phenotype and certain mycobacterial lineages are associated with extrapulmonary TB,[9] although these associations have been inconsistent.[9 10]

Recent epidemiological studies in high-income low-burden Northern Hemisphere countries (including the USA, UK and Germany) have revealed a disproportionate incidence of extrapulmonary TB in ethnic minority populations from high-burden regions.[1 2 11] Indeed, ethnicity appears to be strongly associated with extrapulmonary TB independently of mycobacterial lineage.[12] We postulated that a detailed international epidemiological analysis of clinical disease phenotype encompassing diverse ethnic groups and immigrants from high-burden to low-burden regions might delineate hitherto unrecognised host or environmental factors that influence extrapulmonary dissemination and clinical disease spectrum.

METHODS

Study design and description of data sources

We used routine anonymous TB surveillance data prospectively notified in two independent high-

To cite: Pareek M, Innes J, Sridhar S, *et al*. *Thorax* 2015;**70**:1171–1180.

income settings: Birmingham/Solihull, UK and US Centers for Disease Control (CDC).

Birmingham/Solihull has a population of 1.22 million[13] and between 2007 and 2009 (for Birmingham and Solihull, respectively) the 3-year average TB notifications were 441 and 12, while incidence was 42 and 6 (per 100 000 population per year), respectively.[14] All TB cases are recorded in a centralised chest clinic database allowing ascertainment of all notified cases since 1980.

To confirm that patterns found during the study were not purely UK specific, we analysed data from an independent US dataset—the publicly-available US TB surveillance dataset (Online Tuberculosis Information System—OTIS; http://wonder.cdc.gov/tb.html) which holds aggregate data (individual level information is not publicly available) for all verified cases of active TB in the USA from 1993 to 2008.[15]

Study populations and data collection

We included all cases of active TB diagnosed between 1 January 1980–31 December 2009 (Birmingham/Solihull) and 1 January 1993–31 December 2008 (US CDC) with complete data on age, sex, country of birth (whether local-born or foreign-born), ethnicity and site of disease; patients with missing information for one or more of these variables were excluded from analysis. Further information gathered from the datasets is presented in the online supplementary methods (SI appendix).

Measurement of 25(OH)D in a separate UK TB cohort

We included all consecutively diagnosed cases of active TB recruited between 1 November 2007 and 30 June 2011 (London) and 1 January 2010 and 10 July 2012 (Leicester; with complete demographic and clinical data). We included those patients where, depending on the treating physician's practice, a serum 25(OH)D sample had been drawn up to 90 days prior to diagnosis or within 30 days of treatment initiation.

Definitions of patterns of tuberculous disease

Active TB cases were defined by the major sites of disease into three distinct clinical patterns: pulmonary-only (only lung parenchyma involved), extrapulmonary-only (only one or more extrapulmonary site(s) involved) and both pulmonary and extrapulmonary (pulmonary disease and one or more extrapulmonary sites involved concurrently). Further details of the definitions of different patterns of disease are outlined in the online supplementary methods (SI appendix).

Data analysis

Data analysis was conducted in several steps. Continuous data were summarised with median and IQR and compared using the non-parametric Mann–Whitney U test. Categorical responses were expressed as a percentage and comparisons made using Pearson's χ^2 test (or Fisher's exact test if appropriate).

For the Birmingham and CDC datasets, we assessed temporal trends in extrapulmonary TB diagnosis (using χ^2 test for trend) and compared the numbers (and proportion) of cases of each disease type stratified by local/foreign-birth and white/non-white ethnicity (using Fisher's exact test). Univariable analysis of factors associated with extrapulmonary TB only was assessed using logistical regression and reported as crude ORs and 95% CIs. To calculate adjusted OR (and 95% CI), we mutually adjusted for year, and season, of notification, age, gender, ethnicity, country of birth, time since arrival and HIV status (see online SI appendix for details of regression modelling).

For the London and Leicester datasets, we compared individuals with pulmonary and extrapulmonary disease using a one-way analysis of variance (on the log-transformed concentrations) with Bonferroni correction, if appropriate. The proportions of subjects who were severely vitamin D deficient (serum 25(OH)D <20 nmol/L) were compared between groups using Fisher's exact test. We further assessed the factors associated with extrapulmonary TB in this dataset using logistical regression and reported results as ORs (with 95% CIs). In the multivariable model we adjusted for centre (ie, London or Leicester), year, and season, of notification, age, gender, ethnic group, location of birth, time since arrival, HIV status and serum 25(OH)D concentration. Serum 25(OH)D was used as a continuous variable transformed by $\log_2$; each unit change in $\log_2$ serum 25(OH)D corresponds to a doubling in serum 25(OH)D on the original scale which thereby allows a more clinically relevant, and intuitive, interpretation of the ORs (see SI appendix for further details of logistical regression modelling).

Details of analytical methods are presented in the online supplementary methods (SI appendix). All analyses used STATA V.9.2 (StataCorp, Texas, USA). All tests were two tailed; p values ≤0.05 were considered significant.

RESULTS

Description of cohorts

Study population flow charts for the Birmingham and US CDC cohorts are outlined in the online supplementary figures S1A and S1B, respectively.

Demographic characteristics of the Birmingham and US CDC cohort are set out in table 1. During the Birmingham study period (1980–2009), pulmonary-only disease decreased by 26.1%, while extrapulmonary-only disease increased by 96.6% (p<0.001); the proportion of cases that were exclusively extrapulmonary (compared with pulmonary-only) therefore increased from 31.6% to 52.9% (χ^2 for trend <0.001). During the CDC study period (between 1993–1996 and 2005–2008), overall TB notifications decreased by 43.2% but the decrease in pulmonary only cases (46.5%) was greater than for extrapulmonary-only cases (27.8%). Consequently, the proportion of cases that were exclusively extrapulmonary (as compared with pulmonary-only) increased from 17.8% to 22.6% (χ^2 for trend p<0.001).

Relationship of clinical disease phenotype with foreign-birth and ethnicity

Figure 1 outlines, for the Birmingham and US datasets, the proportion of cases that are pulmonary and extrapulmonary in different demographic groups.

Individuals of UK-born white ethnicity had a significantly lower proportion of extrapulmonary-only disease (18.1%) as compared with individuals of UK-born non-white ethnicity (34.3%; p<0.001) and both had a significantly lower proportion of extrapulmonary disease when compared with individuals of foreign-born non-white ethnicity (49.9%; p<0.001 for UK-born white and UK-born non-white). Similarly in the USA, US-born individuals of white ethnicity had a lower proportion of extrapulmonary-only disease (14.7%) compared with individuals of US-born non-white ethnicity (18.9%; p<0.001) and both groups had a lower proportion of extrapulmonary disease compared with foreign-born non-white cases (24.1%; p<0.001 for US-born white and US-born non-white).

Other factors associated with extrapulmonary TB

In the UK (table 2), multivariable analysis revealed that extrapulmonary TB was associated with increasing age up to 45 years

Table 1 Demographic characteristics of individuals with active TB in the Birmingham and US CDC datasets

Variable	Birmingham (n=10 152)	Variable	CDC USA (n=277 013)
Age (median, IQR)	35.0 (23.0–55.0)		
Age categories		Age categories	
<16	1264 (12.5%)	<5	10 217 (3.7%)
16–25	1960 (19.3%)	5–14	7123 (2.6%)
26–35	1990 (19.6%)	15–24	25 534 (9.2%)
36–45	1358 (13.4%)	25–44	97 349 (35.1%)
46–55	1101 (10.6%)	45–64	76 262 (27.5%)
over 55	2479 (24.4%)	>64	60 528 (21.9%)
Gender		Gender	
Female	4902 (48.3%)	Male	173 621 (62.7%)
Male	5250 (51.7%)	Female	103 392 (37.3%)
Ethnicity		Ethnicity	
White	1991 (19.6%)	American Indian or Alaska Native	3532 (1.3%)
Indian Subcontinent	6233 (61.4%)	Asian or Pacific Islander	56 962 (20.6%)
Afro-Caribbean	1484 (14.6%)	Black or African American	86 697 (31.3%)
Oriental/other Asia	220 (2.2%)	Hispanic or Latino	66 934 (24.2%)
Other	224 (2.2%)	White, non-Hispanic	62 888 (22.7%)
Place of birth		Place of birth	
UK	3612 (35.6%)	USA	153 598 (55.4%)
Foreign-born	6540 (64.4%)	Foreign-born	123 415 (44.6%)
Time since entry to the UK (years)*		Time since entry to the US (years)†	
<1	331 (5.7%)	<1	23 897 (23.7%)
1–5	1943 (33.3%)	1–4	24 990 (24.8%)
6–10	818 (14.0%)	5–14	27 467 (27.3%)
>10	2740 (47.0%)	15+	24 280 (24.1%)
Employment status‡		Employment status§	
Unemployed	447 (11.4%)	Unemployed	144 308 (59.0%)
Retired	334 (8.5%)	Employed	100 345 (41.0%)
Employed/housewife	1670 (42.4%)		
Student/child	1486 (37.7%)		
HIV status¶		HIV status**	
Negative	171 (88.6%)	Negative	101 606 (77.9%)
Positive	22 (11.4%)	Positive	28 836 (22.1%)
Previous TB††		Previous TB‡‡	
No	4777 (95.3%)	No	260 527 (94.8%)
Yes	236 (4.7%)	Yes	14 350 (5.2%)
Type of disease		Type of disease	
Pulmonary only	5590 (55.1%)	Pulmonary only	203 449 (73.4%)
Pulmonary and extrapulmonary	763 (7.5%)	Pulmonary and extrapulmonary	21 524 (7.8%)
Extrapulmonary only	3799 (37.4%)	Extrapulmonary only	52 040 (18.8%)
Site of extrapulmonary disease			
Intrathoracic lymph node	525 (11.5%)		
Extrathoracic lymph node	1907 (41.8%)		
Bone and joint	461 (10.1%)		
Central nervous system	169 (3.7%)		
Pleura	505 (11.7%)		
Peritoneum	108 (2.4%)		
Genitourinary	180 (4.0%)		
Intestinal	212 (4.7%)		
Miliary	198 (4.3%)		
Pericardial	51 (1.1%)		
Soft tissue	101 (2.2%)		
Other	538 (11.8%)		
Culture positive§§		Culture positive¶¶	
No	5398 (53.2%)	No	56 072 (20.2%)
Yes	4754 (46.8%)	Yes	220 941 (79.8%)

Continued

Table 1 Continued

Variable	Birmingham (n=10 152)	Variable	CDC USA (n=277 013)
Drug sensitivity***		Drug sensitivity†††	
Fully sensitive	1634 (91.8%)	Not multidrug resistant	206 274 (98.5%)
Monodrug resistant	116 (6.5%)	Multidrug resistant	3154 (1.5%)
Multidrug resistant	30 (1.7%)		
		Resident in correctional facility‡‡‡	
		No	264 422 (96.3%)
		Yes	10 237 (3.7%)

*Only applies to foreign-born individuals; data available for 5832 individuals.
†Only applies to foreign-born individuals; unknown for 22 781.
‡Data available for 3937 individuals.
§Unknown for 32 360.
¶Data available for 193 individuals.
**Unknown for 146 571.
††Data available for 5013 individuals.
‡‡Unknown for 2136.
§§Culture positive for 3134/5590 (56.1%) pulmonary only cases, 1246/2553 (32.8%) extrapulmonary only cases and 374/763 (49.0%) pulmonary and extrapulmonary cases.
¶¶Culture positive for 165 319/203 449 (81.3%) pulmonary only cases, 36 994/52 040 (71.1%) extrapulmonary only cases and 18 628/21 524 (86.5%) pulmonary and extrapulmonary cases.
***Data available for 1780 individuals.
†††Only relevant to culture positive cases; unknown for 11 513.
‡‡‡Unknown for 2354.
CDC, Centers for Disease Control.

and female gender. In a sensitivity analysis of the UK data, including concurrent pulmonary and extrapulmonary case as extrapulmonary TB did not change the outcomes of the multivariable analysis (data not shown). Univariable analysis of the US population (see online supplementary table S1) revealed that extrapulmonary TB was associated with increasing age up to 45 years, female gender, employment status, HIV status (HIV-seropositivity), no history of TB and drug-sensitive disease.

In both the UK and the USA, as time between arrival and development of active TB increased, the proportion of foreign-born cases that were extrapulmonary doubled and then stabilised (from 24.9% at <1 year to 51.8% at >1 year in Birmingham and from 13.3% at <1 year to 26.8% at >1 year in the USA; p<0.001 for both Birmingham and the USA). Interestingly, foreign-born non-white individuals developing active TB within 12 months of arrival actually had a lower proportion of extrapulmonary TB (24.9% in Birmingham and 13.3% in the USA) than local-born non-whites (34.4% in Birmingham and 18.9% in the USA; p=0.001 and p<0.001 for Birmingham and US CDC, respectively).

Association of serum 25(OH)D level with TB disease phenotype

Given the associations we found between disease pattern and different demographic and ethnic groups, we postulated that vitamin D deficiency may be a key factor determining clinical phenotype. We therefore tested this hypothesis in a clinically precisely defined, ethnically diverse, cohort of 462 patients with TB from London and Leicester, UK (see online supplementary figure S2). Demographic details of the cohort are presented in the online supplementary table S2. Subjects included in the vitamin D cohort were younger and less likely to have HIV coinfection than those subjects excluded from the study due to lack of vitamin D data at diagnosis but were similar in all other respects (see online supplementary table S2).

Overall, mean serum 25(OH)D levels in the cohort were 12.6 nmol/L (95% CI 11.8 to 13.5 nmol/L); 70.4% and 97.8% of the cohort had serum 25(OH)D levels <20 and <75 nmol/L, respectively. Individuals with purely extrapulmonary TB (11.4 nmol/L; 95% CI 10.5 to 12.5 nmol/L) and any extrapulmonary TB (11.4 nmol/L; 95% CI 10.6 to 12.3 nmol/L) had significantly lower serum 25(OH)D levels as compared with individuals with pulmonary TB only (15.2 nmol/L; 95% CI 13.5 to 17.2 nmol/L; p<0.001 and p<0.001, respectively). Severe vitamin D deficiency (serum 25(OH)D <20 nmol/L)[16] was significantly more common in individuals with purely extrapulmonary TB (74.7%; p=0.005) and individuals with any extrapulmonary involvement (75.4%; p=0.001) than in subjects with purely pulmonary TB (60.9%; see online supplementary figure S3). Among foreign-born non-white individuals who were UK-resident for >1 year, those who presented with purely extrapulmonary (10.7 nmol/L; 95% CI 9.8 to 11.6 nmol/L) or any extrapulmonary TB (10.7 nmol/L; 95% CI 9.9 to 11.5 nmol/L) had lower mean serum 25(OH)D levels than those with purely pulmonary TB (12.7 nmol/L; 95% CI 14.7 to 24.5 nmol/L; p=0.030 and p=0.020, respectively).

On multivariable analysis (table 3), a doubling in serum 25(OH)D was associated with a reduced risk of purely extrapulmonary (OR 0.55, 95% CI 0.41 to 0.73) and any extrapulmonary TB (OR 0.66, 95% CI 0.51 to 0.84). Additional factors independently associated with extrapulmonary disease included season of notification (highest in winter/spring when 25(OH)D levels are usually lowest[16 17]), increasing age and, for the foreign-born, increasing time since arrival in the UK. Notably, while on univariable analysis non-white ethnicity was associated with extrapulmonary disease, on multivariable analysis, when taking serum 25(OH)D into account, the association with ethnicity disappeared. In a further sensitivity analysis, results remained quantitatively unchanged when analyses were restricted to those individuals who had had a vitamin D level measured within 30 days of commencing anti-tuberculous therapy (either before or after).

DISCUSSION

In two independent Northern hemisphere settings, clinical patterns of TB significantly and consistently differed by demographic and ethnic group in Northern latitudes. Collectively,

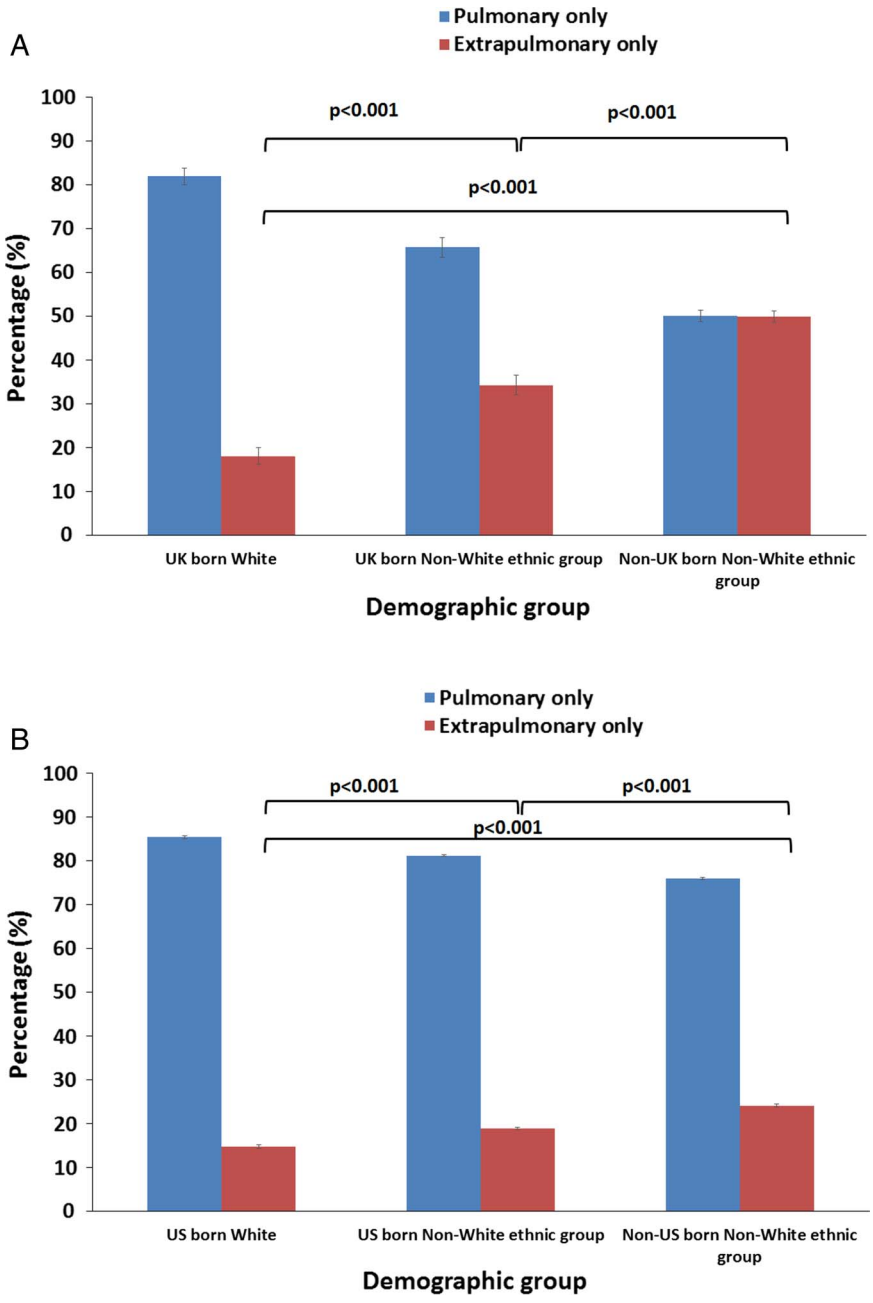

Figure 1 Patterns of TB stratified by location of birth (local or foreign) and ethnic group (white vs non-white) for Birmingham and US Centers for Disease Control (CDC) datasets. [1]Proportions are calculated on purely pulmonary and extrapulmonary cases only (ie, concurrent pulmonary and extrapulmonary cases are excluded for the purposes of this figure). [2]For the Birmingham dataset, concurrent pulmonary and extrapulmonary case numbers (and proportions) were local-born white 72 (4.1%), local-born non-white 136 (7.2%) and foreign-born non-white 547 (8.7%). [3]For the US CDC dataset, concurrent pulmonary and extrapulmonary case numbers (and proportions) were local-born white 3075 (5.6%), local-born non-white 8939 (9.1%) and foreign-born non-white 9009 (7.8%).

these findings suggest an environmental factor influencing clinical phenotype. The internationally and intranationally reproducible relationship of disease phenotype with foreign birth and non-white ethnicity potentially implicates vitamin D status. To directly test this possibility, we analysed serum 25(OH)D concentrations in an independent UK cohort and found that vitamin D deficiency, after adjusting for ethnicity and migration, was significantly associated with extrapulmonary TB. Our data potentially implicate an environmental factor (vitamin D) other than HIV-induced or iatrogenic immunosuppression influencing TB disease phenotype. Previous studies have found associations between sun exposure,[18] seasonality[19 20] and TB incidence as well as between vitamin D deficiency and prevalence of latent[21] and active TB.[5] However, vitamin D deficiency has not hitherto been implicated in determining clinical phenotype. Despite vitamin D's pivotal role in antimycobacterial host defence[22–26] and the augmentation of antimycobacterial immunity with vitamin D supplementation,[27] the clinical effects of vitamin D deficiency in TB are unknown. We identify extrapulmonary TB as a potential clinical correlate of vitamin D deficiency.

The known associations between certain phylogenetic mycobacterial lineages and particular ethnic groups and disease sites might in theory have confounded our observations as the lineages of the infecting strains in our study populations were not known. However, in a subset of over 1000 patients with TB in the Birmingham cohort where the lineages of the infecting strains were known, non-white ethnicity was strongly and independently associated with extrapulmonary TB on multivariable analysis after taking mycobacterial lineage into account.[12] In the current study, the fact that vitamin D deficiency was associated with extrapulmonary TB independently of non-white ethnicity (which was not associated with extrapulmonary disease after taking vitamin D status into account) suggests that vitamin D deficiency may mediate the widely recognised association of non-white ethnicity with extrapulmonary dissemination.

Table 2 Demographic, clinical and temporal variables associated with different patterns of TB for Birmingham dataset

	Whole cohort (n=9389)*					Non-white ethnic groups (n=7478)*			
Variable	**Extrapulmonary TB only* (n=3799)**	**Unadjusted OR (95% CI)**	**Adjusted OR† (95% CI)**	**p Value**	**Variable**	**Extrapulmonary TB only* (n=3460)**	**Unadjusted OR (95% CI)**	**Adjusted OR‡ (95% CI)**	**p Value**
Period					Period				
1980–1984	568/1798 (31.6)	1	1	<0.001	1980–1984	487/1291 (37.7)	1	1	<0.001
1985–1989	487/1345 (36.2)	1.23 (1.06 to 1.43)	1.24 (1.06 to 1.46)		1985–1989	426/1005 (42.4)	1.21 (1.03 to 1.44)	1.28 (1.08 to 1.53)	
1990–1994	494/1471 (33.6)	1.09 (0.95 to 1.27)	1.09 (0.94 to 1.28)		1990–1994	431/1120 (38.5)	1.03 (0.88 to 1.22)	1.09 (0.92 to 1.3)	
1995–1999	475/1242 (38.2)	1.34 (1.15 to 1.56)	1.28 (1.09 to 1.50)		1995–1999	444/1001 (44.4)	1.32 (1.11 to 1.56)	1.41 (1.18 to 1.68)	
2000–2004	755/1604 (47.1)	1.93 (1.67 to 2.21)	1.85 (1.60 to 2.15)		2000–2004	696/1348 (51.6)	1.76 (1.51 to 2.06)	1.96 (1.67 to 2.31)	
2005–2009	1020/1929 (52.9)	2.43 (2.13 to 2.78)	2.24 (1.94 to 2.59)		2005–2009	976/1713 (57.0)	2.19 (1.89 to 2.53)	2.34 (2.01 to 2.74)	
Season					Season				
Winter	818/2118 (38.6)	1	1	0.370	Winter	732/1661 (44.1)	1	1	0.170
Spring	956/2404 (39.8)	1.05 (0.93 to 1.18)	0.99 (0.87 to 1.12)		Spring	876/1908 (45.9)	1.08 (0.94 to 1.23)	1.01 (0.88 to 1.16)	
Summer	1062/2581 (41.2)	1.11 (0.99 to 1.25)	1.04 (0.92 to 1.18)		Summer	979/2075 (47.2)	1.13 (1.00 to 1.29)	1.11 (0.97 to 1.27)	
Autumn	963/2286 (42.1)	1.16 (1.03 to 1.31)	1.09 (0.96 to 1.24)		Autumn	873/1834 (47.6)	1.15 (1.01 to 1.32)	1.14 (0.99 to 1.31)	
Age categories					Age categories				
<16	383/1167 (32.8)	1	1	<0.001	<16	334/958 (34.9)	1	1	<0.001
16–25	652/1828 (35.7)	1.13 (0.97 to 1.33)	0.80 (0.68 to 0.94)		16–25	634/1678 (37.8)	1.13 (0.96 to 1.34)	0.86 (0.72 to 1.03)	
26–35	923/1840 (50.2)	2.06 (1.77 to 2.40)	1.34 (1.13 to 1.60)		26–35	889/1651 (53.8)	2.18 (1.85 to 2.57)	1.42 (1.18 to 1.72)	
36–45	623/1240 (50.2)	2.07 (1.75 to 2.44)	1.49 (1.23 to 1.80)		36–45	579/999 (58.0)	2.58 (2.15 to 3.09)	1.70 (1.37 to 2.10)	
46–55	468/1022 (45.8)	1.73 (1.45 to 2.06)	1.31 (1.07 to 1.60)		46–55	419/770 (54.4)	2.23 (1.84 to 2.71)	1.47 (1.17 to 1.85)	
Over 55	750/2292 (32.7)	1.00 (0.86 to 1.16)	0.81 (0.68 to 0.98)		Over 55	605/1422 (42.5)	1.38 (1.17 to 1.64)	0.83 (0.67 to 1.03)	
Gender					Gender				
Male	1716/4831 (35.5)	1	1	<0.001	Male	1541/3622 (42.6)	1	1	<0.001
Female	2083/4558 (45.7)	1.53 (1.41 to 1.66)	1.43 (1.31 to 1.56)		Female	1919/3856 (46.0)	1.34 (1.22 to 1.47)	1.37 (1.24 to 1.50)	
Country of birth/ethnicity					Country of birth/time since entry				
UK-born white	301/1663 (18.1)	1	1	<0.001	UK-born	598/1741 (34.3)	1	1	<0.001
UK-born Indian Subcontinent	421/1180 (35.7)	2.51 (2.11 to 2.98)	2.29 (1.89 to 2.78)		Foreign-born/<1 year	77/309 (24.9)	0.63 (0.48 to 0.84)	0.61 (0.45 to 0.81)	
UK-born Afro-Caribbean	156/505 (30.9)	2.02 (1.61 to 2.54)	1.81 (1.43 to 2.30)		Foreign-born/1–5 years	931/1763 (52.8)	2.14 (1.87 to 2.45)	1.87 (1.61 to 2.18)	
UK-born Oriental/other Asia	3/13 (23.1)	1.36 (0.37 to 4.96)	1.10 (0.30 to 4.10)		Foreign-born/6–10 years	405/733 (55.3)	2.36 (1.98 to 2.81)	1.98 (1.63 to 2.42)	
UK-born other	18/43 (41.9)	3.26 (1.76 to 6.05)	2.91 (1.54 to 5.50)		Foreign-born/>10 years	1169/2340 (50.0)	1.91 (1.68 to 2.17)	1.64 (1.39 to 1.94)	
Foreign-born white	38/248 (15.3)	0.82 (0.57 to 1.18)	0.94 (0.65 to 1.36)		Foreign-born/time not known	280/592 (47.3)	1.72 (1.42 to 2.07)	1.67 (1.34 to 2.07)	
Foreign-born Indian Subcontinent	2308/4544 (50.8)	4.67 (4.07 to 5.36)	4.10 (3.56 to 4.73)						
Foreign-born Afro-Caribbean	390/836 (46.7)	3.96 (3.29 to 4.76)	2.78 (2.29 to 3.38)						
Foreign-born Oriental/other Asia	80/194 (41.2)	3.18 (2.32 to 4.34)	2.78 (2.01 to 3.83)						
Foreign-born other	84/163 (51.5)	4.81 (3.45 to 6.70)	3.82 (2.72 to 5.37)						

Continued

Table 2 Continued

Variable	Whole cohort (n=9389)* Extrapulmonary TB only* (n=3799)	Unadjusted OR (95% CI)	Adjusted OR† (95% CI)	p Value	Variable	Non-white ethnic groups (n=7478)* Extrapulmonary TB only* (n=3460)	Unadjusted OR (95% CI)	Adjusted OR‡ (95% CI)	p Value
					Ethnicity§				
					Indian Subcontinent	2729/5724 (47.7)	1	1	<0.001
					Afro-Caribbean	546/1341 (40.7)	0.75 (0.67 to 0.85)	0.69 (0.60 to 0.78)	
					Oriental/Other Asia/ Other	185/413 (44.8)	0.89 (0.73 to 1.09)	0.83 (0.67 to 1.02)	
Employment status					Employment status				
Unemployed	167/401 (41.6)	1			Unemployed	161/325 (49.5)	1		
Retired	106/292 (36.3)	0.80 (0.59 to 1.09)			Retired	86/210 (41.0)	0.71 (0.50 to 1.00)		
Employed/housewife	819/1528 (53.6)	1.62 (1.30 to 2.02)			Employed/housewife	795/1393 (57.1)	1.35 (1.06 to 1.72)		
Student/child	459/1365 (33.6)	0.71 (0.57 to 0.89)			Student/child	410/1149 (35.7)	0.57 (0.44 to 0.72)		
Previous TB					Previous TB				
No	2092/4321 (48.4)	1			No	1971/3700 (53.3)	1		
Yes	81/215 (37.7)	0.64 (0.49 to 0.85)			Yes	76/181 (42.0)	0.63 (0.47 to 0.86)		
HIV status					HIV status				
Negative/unknown	3793/9375 (40.5)	1	1	0.260	Negative/unknown	3454/7464 (46.3)	1	1	0.230
Positive	6/14 (42.9)	1.10 (0.38 to 3.18)	0.54 (0.18 to 1.59)		Positive	6/14 (42.9)	0.87 (0.30 to 2.51)	0.51 (0.17 to 1.52)	
Drug sensitivity					Drug sensitivity				
Fully sensitive	585/1452 (40.3)	1			Fully sensitive	566/1244 (45.5)	1		
Monoresistant	44/110 (40.0)	0.99 (0.67 to 1.47)			Monoresistant	44/100 (44.0)	0.94 (0.62 to 1.42)		
Multidrug resistant	7/26 (26.9)	0.55 (0.23 to 1.31)			Multidrug resistant	7/19 (36.8)	0.70 (0.27 to 1.79)		

*Comparison of extrapulmonary TB cases only versus pulmonary TB only (concurrent pulmonary–extrapulmonary cases excluded).
†Models mutually adjusted for year of notification, season of notification, age, gender, country of birth, ethnicity and HIV status.
‡Model mutually adjusted for year of notification, season of notification, age, gender, country of birth, time since arrival, ethnicity and HIV status.
§For the model restricted to non-white individuals, due to small numbers of individuals in the other category we collapsed together oriental/other Asian with the other category.

Table 3 Demographic, clinical and temporal variables associated with different patterns of TB for the London and Leicester (vitamin D) cohort (n=462)

Variable	Extrapulmonary TB only* (n=217)	Unadjusted OR (95% CI)	Adjusted OR† (95% CI)	p Value	Variable	Any extrapulmonary involvement (n=301)	Unadjusted OR (95% CI)	Adjusted OR† (95% CI)	p Value
Centre					Centre				
London	63/102 (61.8)	1	1	0.050	London	82/121 (67.8)	1	1	0.140
Leicester	154/276 (55.8)	0.78 (0.49 to 1.24)	0.42 (0.18 to 0.99)		Leicester	219/341 (64.2)	0.85 (0.55 to 1.33)	0.54 (0.24 to 1.21)	
Period of notification					Period of notification				
2007–2009	37/64 (57.8)	1	1	0.590	2007–2009	48/75 (64.0)	1	1	0.600
2010–2012	180/314 (57.3)	0.98 (0.57 to 1.69)	1.31 (0.49 to 3.51)		2010–2012	253/387 (65.4)	11.06 (0.63 to 1.78)	11.28 (0.51 to 3.24)	
Season					Season				
Winter	51/82 (62.3)	1	1	<0.001	Winter	72/103 (69.9)	1	1	0.003
Spring	76/124 (61.3)	0.96 (0.54 to 1.71)	0.88 (0.44 to 1.75)		Spring	104/152 (68.4)	0.93 (0.54 to 1.60)	0.81 (0.43 to 1.52)	
Summer	68/115 (59.1)	0.88 (0.49 to 1.57)	1.11 (0.55 to 2.25)		Summer	94/141 (66.7)	0.86 (0.50 to 1.49)	1.00 (0.52 to 1.93)	
Autumn	22/57 (38.6)	0.38 (0.19 to 0.77)	0.24 (0.10 to 0.55)		Autumn	31/65 (47.0)	0.38 (0.20 to 0.72)	0.30 (0.14 to 0.62)	
Age categories, n (%)					Age categorical, n (%)				
16–25	32/79 (40.5)	1	1	0.005	16–25	54/101 (53.5)	1	1	0.040
26–35	64/115 (55.7)	1.84 (1.03 to 3.29)	1.90 (0.95 to 3.78)		26–35	93/144 (64.6)	1.59 (0.94 to 2.67)	1.53 (0.83 to 2.81)	
36–45	54/77 (70.1)	3.44 (1.77 to 6.69)	4.28 (1.87 to 9.81)		36–45	64/87 (73.6)	2.42 (1.31 to 4.49)	2.81 (1.32 to 5.99)	
46–55	31/50 (62.0)	2.40 (1.16 to 4.96)	4.08 (1.59 to 10.47)		46–55	44/63 (69.8)	2.02 (1.04 to 3.92)	2.64 (1.14 to 6.08)	
Over 55	36/57 (63.2)	2.52 (1.25 to 5.08)	4.14 (1.56 to 11.02)		Over 55	46/67 (68.7)	1.91 (0.99 to 3.64)	3.06 (1.26 to 7.43)	
Gender					Gender				
Male	110/208 (52.9)	1	1	0.063	Male	165/263 (62.7)	1	1	0.160
Female	107/170 (62.9)	1.51 (1.00 to 2.29)	1.62 (0.97 to 2.69)		Female	136/199 (68.3)	1.28 (0.87 to 1.89)	1.40 (0.88 to 2.24)	
Country of birth/time since entry					Country of birth/time since entry				
UK-born	21/62 (33.9)	1	1	0.003	UK-born	24/65 (36.9)	1	1	0.001
Foreign-born/<1 year	4/13 (30.8)	0.87 (0.24 to 3.15)	0.88 (0.19 to 4.13)		Foreign-born/<1 year	5/14 (35.7)	0.95 (0.28 to 3.16)	0.68 (0.15 to 2.95)	
Foreign-born/ 1–5 years	59/101 (58.4)	2.74 (1.42 to 5.30)	2.71 (1.19 to 6.14)		Foreign-born/1–5 years	99/141 (70.2)	4.03 (2.17 to 7.48)	3.75 (1.77 to 7.95)	
Foreign-born/ 6–10 years	50/71 (66.3)	4.65 (2.23 to 9.67)	4.76 (1.95 to 11.64)		Foreign-born/6–10 years	58/79 (73.4)	4.72 (2.32 to 9.59)	4.07 (1.75 to 9.45)	
Foreign-born/>10 years	73/107 (68.2)	4.19 (2.16 to 8.15)	2.42 (1.04 to 5.66)		Foreign-born/>10 years	99/133 (74.4)	4.97 (2.63 to 9.40)	2.84 (1.28 to 6.30)	
Foreign-born/time not known	3/11 (27.3)	0.73 (0.18 to 3.05)	0.51 (0.11 to 2.90)		Foreign-born/time not known	8/16 (50.0)	1.71 (0.57 to 5.14)	1.53 (0.43 to 5.46)	
Ethnicity					Ethnicity				
White	18/51 (35.3)	1	1	0.650	White	19/52 (36.5)	1	1	0.170
Indian Subcontinent	136/224 (60.7)	2.83 (1.50 to 5.34)	1.53 (0.64 to 3.67)		Indian Subcontinent	188/276 (68.1)	3.71 (2.00 to 6.89)	2.21 (0.97 to 5.02)	
Afro-Caribbean	33/52 (63.5)	3.18 (1.42 to 7.12)	1.43 (0.51 to 4.02)		Afro-Caribbean	46/65 (70.8)	4.20 (1.93 to 9.15)	1.99 (0.76 to 5.22)	
Oriental/other Asia/other	30/51 (58.8)	2.62 (1.18 to 5.83)	1.94 (0.69 to 5.39)		Oriental/other Asia/other	48/69 (69.6)	3.97 (1.85 to 8.51)	2.88 (1.11 to 7.45)	
Vitamin D status					Vitamin D				
25(OH)D level (log_2 nmol/L)‡		0.68 (0.55 to 0.83)	0.55 (0.41 to 0.73)	<0.001	Vitamin D level (log_2 nmol/L)		0.68 (0.56 to 0.82)	0.66 (0.51 to 0.84)	0.001
HIV status					HIV status				
Negative	198/354 (55.9)	1	1	0.350	Negative	274/430 (63.7)	1	1	0.520
Positive	5/7 (71.4)	1.97 (0.38 to 10.29)	2.59 (0.36 to 18.69)		Positive	6/8 (75.0)	1.71 (0.34 to 8.56)	1.87 (0.28 to 12.74)	

*Comparison of extrapulmonary TB cases-only versus pulmonary TB-only (concurrent pulmonary–extrapulmonary cases excluded).
†Models mutually adjusted for period of notification, season of notification, age, gender, country of birth/time since entry, ethnicity, 25(OH)D level and HIV status.
‡Refers to the OR associated with an increase in continuous log-transformed 25(OH)D levels by log_2 (which corresponds to a doubling in 25(OH)D levels).

Although men are more likely to present with TB per se, we found that extrapulmonary TB was associated with female gender, as previously observed.[2 11] Vitamin D deficiency is more common in women[17] and, interestingly, the association of female gender with extrapulmonary TB disappeared after taking vitamin D status into account on multivariable analysis.

Although seasonality of TB incidence has previously been reported,[19] this is the first study to assess TB seasonality in a population with known 25(OH)D status, enabling us to independently assess the influence of these two factors on disease phenotype. The proportion of extrapulmonary to pulmonary TB was highly seasonal, being highest in winter and spring (when 25(OH)D status is lowest in Northern Hemisphere residents) and lowest in the autumn (when 25(OH)D status is highest). However, after controlling for 25(OH)D status, extrapulmonary TB incidence remained independently associated with seasonality, suggesting that an additional, as yet undetermined, seasonal factor may influence clinical phenotype.

The intermediate prevalence of extrapulmonary TB in local-born non-whites (between UK-born whites and foreign-born non-whites) is consistent with a role for vitamin D deficiency since UK-born non-whites have intermediate levels of 25(OH)D when compared with UK-born whites and foreign-born non-whites.[28] Interestingly, the increasing prevalence of extrapulmonary TB in the USA, especially in non-white individuals, corresponds to the increasing prevalence of vitamin D deficiency in the USA over time which is also most marked in non-white individuals.[29] Such changes might also explain the temporal trend in Birmingham, although there may be increasing ascertainment with wider use of cross-sectional radiology.

The large sample size, independent northern latitude settings and multiple diverse ethnic groups in this study suggest that our findings are generalisable. Moreover, the size of effect for the association between vitamin D deficiency and extrapulmonary disease is not inconsequential, being higher than, or in line with, ORs associated with pathogen[9] and host genetic[6 8] factors predisposing to extrapulmonary TB.

How vitamin D deficiency preferentially favours development of extrapulmonary TB is unclear and may depend on whether haematogenous spread to extrapulmonary sites occurs after primary infection. If following initial infection, *Mycobacterium tuberculosis* is contained within the lungs, then vitamin D deficiency may cause T-cell immunosuppression or upregulated matrix metalloproteinase activity promoting basement membrane penetration,[30] resulting in impaired bacillary containment predisposing to extrathoracic dissemination and seeding. This would then increase the likelihood of extrapulmonary disease upon subsequent reactivation. Alternatively, if bacilli routinely spread haematogenously after initial pulmonary infection and are then contained by host responses in peripheral extrapulmonary sites as well as in the lung, the mechanisms of peripheral containment may be more vitamin D-dependent than those in the lung. Regardless of which model is correct, our patient-based data support a role for vitamin D in bacillary containment, suggesting that the potent downstream vitamin D-dependent antimycobacterial macrophage effector mechanisms elucidated in vitro might also mediate mycobacterial containment in vivo.[22 25 26]

Our work has several limitations. Data were obtained from surveillance systems limiting our analysis to routinely collected variables. HIV status was incompletely recorded in the surveillance datasets;[2] however, in the separate UK cohort where 25(OH)D was measured, HIV status was known for most patients. Given the US CDC aggregate data, regression modelling was univariable rather than multivariable. In the vitamin D cohort not all patients had vitamin D levels checked or checked in the eligible time period and it is possible that physicians only selected specific patients for testing meaning that we could not completely exclude the possibility of selection bias. We did not have data on possible use of vitamin D supplements. Also, it is possible that serum 25(OH)D concentrations may have been affected by anti-tuberculous treatment in those individuals who had already commenced treatment but any such effect was mitigated by including only patients whose levels were measured within 30 days of treatment initiation. In any event, both these latter factors would have affected all TB phenotypes equally, not specifically extrapulmonary TB. It is possible that the extrapulmonary phenotype was related to some other unmeasured factor (such as socioeconomic or nutritional status), which was in turn correlated both with ethnicity and with vitamin D status. Furthermore, it is possible, although unproven, that patients with extrapulmonary TB might behave differently to patients with pulmonary TB (eg, spending more time indoors) consequently resulting in lower vitamin D levels.

Our classification of TB disease was based on exclusively pulmonary or extrapulmonary involvement. Since extrapulmonary disease is more likely to be culture-negative, it might theoretically have been clinically overdiagnosed in certain demographic groups. However, this is unlikely as our findings were based on complete surveillance datasets and were unaffected by changes in classification of extrapulmonary TB (where extrapulmonary was defined as pulmonary plus extrapulmonary) and by limiting analysis to culture-positive cases only.

While vitamin D deficiency appears to be a risk factor for extrapulmonary TB, our findings do not confirm causality. It is theoretically possible that deficiency is a consequence of unrelated immunological changes that predispose to extrapulmonary TB or instead caused by an extrapulmonary disease phenotype or dissemination (ie, reverse causality). If vitamin D deficiency does play a causal role, the implications for international public health, particularly migrant health, as well as for mechanisms of in vivo containment of *M tuberculosis* could be considerable.

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Contributors AL, MP and SS conceived of the idea for the study. JI, MD, MW and GW collected the clinical data as part of routine care. LG extracted data for the London cohort. MP undertook the data analysis. MP and AL wrote the first draft of the manuscript with subsequent revisions made by all other coauthors. All coauthors had sight of the submitted paper. AL is guarantor for the paper.

Funding MP is an NIHR Academic Clinical Lecturer in Infectious Diseases. His PhD was funded by a Medical Research Council Capacity Building Studentship. AL is a Wellcome Senior Research Fellow in Clinical Science and NIHR Senior Investigator. This work was supported by the NIHR Health Protection Research Unit in Respiratory Infections, Imperial College London. The funding bodies played no role in data collection, analysis or interpretation. They were not involved in the decision to submit the paper for publication. None of the authors have been paid to write this article by a pharmaceutical company or other agency. The corresponding author had

full access to all the data in the study and had final responsibility for the decision to submit for publication.

Competing interests AL is Non-Executive Director of a nutraceutical company several of whose products contain vitamin D. MP, JI, SS, LG, DC, GW, MW, ARM, OMK and MD declare that they have no conflict of interest.

Provenance and peer review Not commissioned; externally peer reviewed.

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