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## Public Health

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## Original Research

# Discordance in latent tuberculosis (TB) test results in patients with end-stage renal disease



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## ARTICLE INFO

## Article history:

Received 7 June 2018

Received in revised form

18 September 2018

Accepted 25 September 2018

Available online 12 November 2018

## Keywords:

Tuberculosis

ESRD

Renal disease

Dialysis

TST

Tuberculin skin test

IGRA

Interferon-gamma release assay

## ABSTRACT

**Objectives:** This natural experiment was designed to assess the impact of exposure to an active case of tuberculosis (TB) on a group of immunosuppressed individuals, with end-stage renal disease over an extended follow-up.

**Study design:** Close contacts of people with sputum smear-positive *Mycobacterium tuberculosis* are at high risk of infection, particularly immunosuppressed individuals. An infectious TB healthcare worker worked in a renal dialysis unit for a month before diagnosis, with 104 renal dialysis patients, was exposed for  $\geq 8$  h.

**Methods:** Patients were informed and invited for screening 8–10 weeks postexposure. They either underwent standard two-step assessment with tuberculin skin test (TST) and QuantiFERON<sup>®</sup>-TB Gold (Cellestis GmbH; QFN) interferon-gamma release assay (IGRA) or after consent, enrolled in a study where these two tests were performed simultaneously with T-SPOT<sup>®</sup>-TB (Oxford Immunotec Ltd; TSPOT). Patients within the study were followed up for 2 years from exposure, with QFN and TSPOT repeated at months 3 and 6 from the first testing.

**Results:** Of 104 exposed individuals, 75 enrolled in the study. There was a high degree of discordance among QFN, TSPOT and TST. This was seen at both the first time point and also over time in subjects who were retested. No patients had active TB at the baseline testing. None received treatment for latent TB infection. Over the following 2 years, no one developed TB disease.

**Conclusion:** This study suggests that there is a low risk of progression to active TB in low-incidence countries even in high-risk groups. This plus the degree of the test result discordance emphasises the complexities of managing TB in such settings as it is unclear which of these tests, if any, provides the best diagnostic accuracy.

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<https://doi.org/10.1016/j.puhe.2018.09.023>

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## Introduction

After exposure to *Mycobacterium tuberculosis* complex (M.tb), a person may develop tuberculosis (TB), regardless of his/her age, gender, race, religion or socio-economic status.<sup>1</sup> However, some groups are at considerably greater risk of progression from infection to disease as they are immunocompromised, including those receiving dialysis. Given that an assessment for M.tb infection relies on measuring host immune response, and this may be dysregulated in the immunocompromised individuals, it can be hard to ascertain immunologically whether someone who has been exposed to M.tb has established infection and therefore is at risk of active TB.<sup>2,3</sup>

Although studies have been conducted to assess how to manage M.tb exposure and the appropriateness of testing for latent TB infection in the high-risk population with end-stage renal disease (ESRD) and receiving renal dialysis,<sup>3–6</sup> little work has compared commercially available tests to determine result consistency between and within tests over time.

A healthcare worker with strongly smear-positive drug-sensitive cavitary pulmonary TB worked full time on a renal dialysis unit in a large London hospital during 2011. Owing to the nature of care required in those receiving renal dialysis, the healthcare worker had prolonged close contact, of 4–5 h on each occasion, with patients during their thrice-weekly visits. Contact would have included attaching and detaching patients from the dialysis machinery, collecting required blood samples and providing general care while they were present at the unit. This exposure provided the opportunity to assess whether patients had been infected shortly after exposure and any conversions or reversions subsequently. We also followed up the participants for 2 years to determine disease outcomes. Our objective was, therefore, to assess the rate of TB infection, test conversion/reversion and progression from latent infection to active disease in patients with ESRD exposed to a healthcare worker with pulmonary TB for a period of up to 4 weeks.

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## Methods

### Population

All exposed patients with ESRD between 31 January 2011 and 26 February 2011 were informed of the incident and were invited for screening by the local TB service. They were also sent information about a study into which they could enrol in place of routine screening. Those who did not wish to take part were managed by the TB service and underwent two-step assessment with tuberculin skin test (TST) and QuantiFERON®-TB Gold (QFN) interferon-gamma release assay (IGRA; Cellestis GmbH at the time of the study, now Qiagen). Those who enrolled in the study were managed as mentioned in the following section. All subjects were assessed for TB disease with a symptom questionnaire, chest radiograph and other tests as indicated.

### Testing regimen

All study participants had blood draws for assessment using the two commercially available IGRAs: QFN and T-SPOT®-TB (TSPOT), at enrolment to the study approximately 8 weeks after exposure and at 3 and 6 months later. All participants also received a TST on enrolment, with a cut-off  $\geq 6$  mm for a TST with no prior *Bacillus Calmette-Guérin* (BCG) vaccination or  $\geq 15$  mm with prior BCG as per the National Institute of Health and Care Excellence guidelines, being considered positive.

QFN was performed at the immunology laboratory, Royal Free Hospital (London), and TSPOT at the department of respiratory medicine, Imperial College (London) or the National Mycobacteria Reference Laboratory of Public Health England. These were performed using the standard commercial kits and associated standard operating procedures for conduct and interpretation of results. Uninterpretable results were those classified as indeterminate, equivocal or borderline according to the test manufacturer specifications and study SOPs.<sup>7,8</sup>

### Data collection

Information on demographics and TB risk factors such as age, gender, country of birth, travel history to high TB burden countries, smoking, drug use and medical history of immunosuppressive illness and social risk factors such as homelessness and prison stays were collected at enrolment by trained research nurses using a standardised questionnaire.

### Analysis

The association of any of the demographic information with positive results for each of the three tests performed on study entry was assessed using Kruskal-Wallis tests, except for the gender distribution which was determined using Fisher's exact test. No regression analyses were undertaken due to the small numbers in subsets of the data.

Concordance between the IGRAs and TST is presented as descriptive data at each of the three testing time points. Consistency over time of the IGRA is illustrated by data from those with information on every test at every time point.

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## Results

Of the 104 patients involved in the exposure incident, 75 enrolled in the head-to-head study. No patient had evidence of TB disease at the baseline assessment.

### Study population

**Table 1** sets out study participants' demographics and risk for TB. The median age of the participants was 66 years (range 23–88 years), 42 (56%) were men and the ethnicity of the group was diverse. Previous BCG was noted in most participants, but all were vaccinated more than 5 years before the exposure incident. The majority (69%) were non-UK born, although all had lived in the UK for 5 years or more, and only three had visited an endemic area in the recent years. Nobody was using

**Table 1 – Demographics and risk factors for tuberculosis in participants categorised by TST and IGRA results.**

Demographic	Total	<sup>a</sup> TST positive	<sup>a</sup> QFN positive	<sup>a</sup> TSPOT-TB positive
N (%)	75	9 (12)	10 (13)	11 (15)
Age, years (Median/range)	66 (23–88)	76 (23–85)	73 (44–83)	74 (23–80)
19–44 [n (%)]	10 (13)	2 (22)	1 (10)	3 (27)
45–64 [n (%)]	27 (36)	1 (11)	1 (10)	2 (18)
≥65 [n (%)]	38 (51)	7 (77)	8 (80)	6 (55)
Gender				
M:F	42:33	6:3	6:4	5:6
Ethnicity [n (%)]				
White	30 (40)	3 (33)	3 (30)	3 (27)
Bangladeshi	1(1)	0	0	0
Black African	10 (13)	3 (33)	0	3 (27)
Black Caribbean	17 (23)	2 (22)	0	1 (9)
Indian	8 (11)	1 (11)	2 (20)	2 (18)
Pakistani	1 (1)	0	1 (10)	0
Mixed/other	6 (8)	0	2 (20)	2 (18)
Unknown	2 (3)	0	1 (10)	0
Country of birth [n (%)]				
UK born	22 (29)	2	0	2
Non-UK born	52 (69)	7	8	8
Not known	1 (1)		2	1
Years in UK [n (%)]				
<2	0	0	0	0
>2 < 5	0	0	0	0
≥5	52 (69)	7	8	8
Not known	0	2	2	3
TB risk factor [n (%)]				
Diabetes [n (%)]	33 (44)	3 (33)	5 (50)	4 (36)
Of which, control:				
Diet	6 (18)	0	0	1 (25)
Tablets	4 (12)	1 (33)	0	0
Injection	18 (55)	1 (33)	3 (60)	2 (50)
Not known	5 (15)	1 (33)	2 (40)	1 (25)
Smoking [n (%)]	34 (45)	5 (56)	6 (60)	6 (67)
BCG [n (%)]				
Yes	45 (60)	6 (66)	4 (40)	9 (82)
No	10 (13)	3 (33)	2 (20)	0
Not known	20 (26)	0	4 (40)	2 (18)
Time BCG given [n (%)]				
≥5 years	37 (82)	5 (83)	3 (75)	5 (55)
Not known	8 (18)	1 (17)	1 (25)	4 (45)
Travelled to endemic area [n (%)]	3 (4)	0	1 (10)	1 (11)
Previous TB contact [n (%)]	7 (9)	0	1 (10)	1 (11)
Previous TB diagnosis [n (%)]	7 (9)	2 (22)	1 (10)	1 (11)
Treated with ≥1 month drug therapy	Yes	Yes	Yes	Yes
HIV positive [n (%)]	1 (1)	0	0	0
Homeless [n (%)]	4 (5)	0	0	0
Of which:				
Current	1 (25)			
>5 years ago	3 (75)			
Prison [n (%)]	0	0	0	0

TB, tuberculosis; TST, tuberculin skin test; IGRA, interferon-gamma release assay; QFN, QuantiFERON; TSPOT, T-SPOT.

Comparisons made were each test positive group vs the remainder of the study cohort—because of the small numbers and for ease of reference, data for the remainder of the cohort are not reported for every measure but are shown for the overall study group.

<sup>a</sup> Kruskal–Wallis tests were used to compare each measure, except gender where Fisher's exact test was used.

biologics such as anti-TNF agents, although three reported taking immunosuppressive drugs. Seven (9%) had undergone a solid organ transplant. Two had had a haematological malignancy; one, a jejunioileal bypass; one, a gastrectomy; and one, silicosis; and all were currently on renal dialysis.

Seven (9%) were previous contacts of TB between 1945 and 2007. Six (8%) people had previously been diagnosed with TB

themselves between 1972 and 2009, including one of the contacts described previously. All had received at least 1 month of drug therapy.

One participant reported being HIV positive, and 33 (44%) were diabetic with 22 using insulin; six, diet control and five, reporting no control measures. One had used illicit drugs in the past 5 years, and 34 (45%) currently smoked tobacco. Four

**Table 2a – IGRA and TST results at study entry, time point zero (71 participants with a TST and at least one IGRA result).**

TST <sup>a</sup>	TSPOT <sup>a</sup>	QFN <sup>a</sup>	Number of participants (%)
Valid results (i.e. positive or negative)			
+	+	+	0
–	–	–	39
+	–	–	4
+	+	–	4
+	–	+	0
–	+	+	0
–	+	–	4
–	–	+	9
At least one invalid result (i.e. equivocal, indeterminate or not tested owing to incorrect blood volume or not testable by test manufacturer instructions)			
–	Borderline positive	Equivocal	1
+	Not tested	Not tested	1
–	Borderline positive	–	2
–	–	Equivocal	2
–	–	Indeterminate	1
–	+	Indeterminate	1
–	+	Not tested	1
–	Not tested	+	1
+	+	Not tested	1

BCG, Bacillus Calmette-Guérin; TST, tuberculin skin test; TSPOT, T-SPOT; QFN, QuantiFERON; IGRA, interferon-gamma release assay; NICE, National Institute of Health and Care Excellence.

<sup>a</sup> Positivity was considered  $\geq 6$  mm for TST with no prior BCG or  $\geq 15$  mm with prior BCG as per NICE guidelines and as per manufacturer-defined outcomes for QFT-G and TSPOT-TB.

reported having been homeless, three within the past 5 years and one was currently homeless. Nobody in the study was, or had previously been, in prison.

#### Latent TB tests stratified by participant characteristics

The median age of those participating was 66 years (range 23–88 years), with the highest proportion with any test positive noted in the  $\geq 65$  year age group: 51% overall with 77% for TST, 80% for QFN and 55% for TSPOT. There was higher positivity in men than in women for TST and QFN but the reverse for TSPOT. Statistical comparison by participant demographic

characteristics and medical history did not reveal any significant differences (Table 1). P values were all  $\geq 0.1$ , so no regression analyses were undertaken.

#### Test results at the baseline, 3 months and 6 months

Blood samples for TSPOT and QFN were collected at enrolment from 73 (97%) participants, 53 (71%) at 3 months later and 52 (69%) at 6 months. All 75 had TST administered and read on entering the study.

At study enrolment, 71 participants had results for at least one IGRA and TST (Table 2a), while 60 participants had a full set of valid results, i.e. positive or negative IGRAs at each time point, followed by 39 participants at 3 months with 12 uninterpretable and 45 participants at 6 months with seven uninterpretable (Table 2a and b). Equivocal results, where the initial result was too close to the manufacturer-set cut-off, prompted a repeat test (where sufficient sample remained) and were the assigned to the appropriate result category or remained equivocal if the repeat was indeterminate. TST was positive at the baseline in 14% of participants and 17% for each of TSPOT and QFN, although not necessarily the same individuals. Most participants were, therefore, negative for all three measures at each time point (Table 2 a and b).

Only 27 participants had both IGRAs at all three time points and a TST on entry (Table 3). Over time, 20 (74%) negative results remained consistent. The seven inconsistent results showed no clear trend in terms of reversion or conversion between the two IGRAs. All seven had the baseline negative TST. For QFT, four participants remained positive at all three time points, while three were consistently negative. For TSPOT, all five participants who were negative at the baseline converted at 3 or 6 months (with one person reverting to negative). Two positive at the baseline reverted.

No participant had evidence of active TB after 2 years of follow-up.

## Discussion

We found that diagnosis of latent TB in this population is challenging, given the lack of a consistent pattern over time or between tests in IGRA results. None of the participants in this study were diagnosed with active TB at the baseline or after 2 years of follow-up. This was surprising, given their immunocompromised status and exposure to the index case over an

**Table 2b – IGRA results at the 3- and 6-month follow-up (NB, no repeat TST was performed at follow-up visits).**

TSPOT	QFN	Number of participants		
		At study start, total = 72 [n (%)]	At 3 months, total = 46 [n (%)]	At 6 months, total = 45 [n (%)]
–	–	43 (60)	33 (72)	36 (80)
+	+	0	3 (7)	4 (9)
+	–	8 (11)	2 (4)	3 (7)
–	+	9 (13)	1 (2)	2 (4)
Excluded because of invalid test, borderline, equivocal or indeterminate results	11	12 (16)	7 (15)	

TST, tuberculin skin test; TSPOT, T-SPOT; QFN, QuantiFERON; IGRA, interferon-gamma release assay.

**Table 3 – Results for all participants with a full set of test results, i.e. valid IGRAs at each time point and a TST with any discordance<sup>a</sup>.**

Subject number	Study entry, time 0			3-month follow-up		6-month follow-up		Consistency over time
	TST	TSPOT	QFN	TSPOT	QFN	TSPOT	QFN	
P00270	–	–	+	+	+	–	+	Inconsistent
P00436	–	–	+	+	+	+	+	Inconsistent
P00218	–	+	–	–	–	–	–	Inconsistent
P00433	–	–	+	+	+	+	+	Inconsistent
P00284	–	–	+	–	+	+	+	Inconsistent
P00381	–	+	–	–	–	–	–	Inconsistent
P00275	–	–	–	–	–	+	–	Inconsistent
Total								Total = 7 Inconsistent

TST, tuberculin skin test; TSPOT, T-SPOT; QFN, QuantiFERON; IGRA, interferon-gamma release assay.

<sup>a</sup> Twenty further participants had consistent results that were negative throughout.

extended period. Possible explanations for this include a low infectivity of the index case or that patient contact was less than we had estimated from nursing and medical records.

As well as the discordance in IGRA results, there was similarly a high level of discordance between the two IGRAs and with TST at study enrolment. Identifying who was infected with *M.tb* was, therefore, particularly difficult, given the inconsistent pattern observed. Furthermore, in the participants with results at multiple time points, TSPOT results converted or reverted over time with no specific pattern. Immunosuppression, older age and origin in a high TB burden all likely contributed to the overall variability and lack of consistent results.

Despite the exposure of these immunosuppressed patients to a smear-positive TB patient, the proportion test positive at the baseline and converting to positive were similar to that observed in the UK-based older migrant populations.<sup>9</sup> Furthermore, no one developed active TB in the absence of preventive treatment for latent TB. Although we do not have results at multiple time points for most participants, the lack of progression to active TB suggests low transmission in this context.

Alternatively, the high proportion of IGRA and TST negative results may reflect an anergic state in this population. This would be inconsistent, however, with the low progression to active TB. The phenomenon of anergy, which is linked with immunosuppression renders the TST, and to a lesser extent IGRA, generally less useful in identifying *M.tb* infection. Studies assessing anergy in patients with ESRD without specific TB exposure, using the definition ‘response <2 mm to candida and/or mumps antigen’, reported 40%<sup>10</sup> and 23% anergy,<sup>11</sup> where a positive TST was noted in 19% and 30%, respectively. A study in California found 14% TST positivity<sup>12</sup>—similar to that in our UK report.

It has also been suggested that anergy and its impact on underlying immunological mechanisms may affect QFN results, which should, therefore, be interpreted with caution in immunosuppressed patients.<sup>3</sup> Such anergy would likely be illustrated by a higher proportion of uninterpretable results compared with that seen in the general population or in an immunocompetent group. This was demonstrated when 62 patients with ESRD in Switzerland were screened, where 29% were TSPOT positive and 8% indeterminate and 8% were QFN positive with 8% indeterminate.<sup>13</sup> It was also observed in our study, with 18% uninterpretable IGRA results according to the test manufacturer specifications at study entry.

A US study in 80 renal dialysis patients tested at a single time point concluded that ‘TST was not helpful in identifying LTBI in this population’.<sup>4</sup> They argued for the superiority of IGRAs over TST reporting a kappa correlation of 0.59 between the IGRA result and conventional diagnosis, defined as a combination of chest X-ray, TST and screening questionnaire, although they found no superiority of one IGRA over the other.

Further support for IGRA compared with TST in populations with ESRD was documented after routine screening of 203 such patients in Canada.<sup>6</sup> TST positivity was reported in 12.8% compared with 35.5% by TSPOT, a greater difference than 14% and 17%, respectively, found in our study. They concluded that TST was insensitive in haemodialysis patients and was not recommended to be used in isolation in diagnosing LTBI. Instead, they recommended that ‘a combination of TSPOT and medical assessment may be the most accurate screening method’, although it was not clear how LTBI should be determined.

Evaluation of QFN in a general population group from New York found 16% positive and <1% indeterminate among 20,000 people.<sup>14</sup> Increasing age and birth in a high incidence country were found to be associated with positive results, in line with those reported here, where patients were generally older adults from high incidence countries, but with the added complications associated with their medical status.

Not surprisingly, given the global incidence of TB and of people with ESRD, exposure incidents similar to those reported here have been documented previously in various countries. None have applied all three diagnostic measures, although similar risk factors for infection were identified. In Spain, 52 patients with ESRD were exposed to a TB-infected nurse for 17 weeks, and 18 (35%) were QFN positive. Three (6%) patients were TST positive, with what they described as weak concordance—having a kappa value of 0.21.<sup>5</sup> This is higher than the 16% identified in the present study, although the exposure period was much longer in the Spanish incident and the infectiousness and close patient contact of the index case may have been greater. The Spanish group found that, when they considered erythema and induration in interpreting the TST, the kappa increased to 0.67. They concluded that QFN was more accurate in diagnosing LTBI in patients with ESRD than TST and that, unconventionally, erythema should be considered when interpreting TST results in this group.

Interpretation of LTBI tests in this patient group is complex, given the demographics of patients with ESRD who are

often older and suffer comorbidities. The context of the incident is also relevant. This includes the background of the patients, who may, as in our study, be immigrants from high TB incidence countries. While in most instances, immunocompromised patients will be expected to be offered preventative treatment after such exposure, the administration of treatment should balance the risk of development of active TB against the addition of potentially toxic medication to already complex medical treatments in this patient population.

## Author statements

### Acknowledgements

The authors are grateful primarily to the study participants for their engagement with this work. They appreciate the cooperation of the staff at the renal unit, colleagues who collected and tested samples and the support team who arranged study logistics.

### Ethical approval

Recruitment and follow-up of participants was through the PREDICT study, of which this group was a subset for this specific exposure incident, which was approved by the Brent Research Ethics Committee (reference 10/H0717/14) and by the R&D Department of the NHS Trust involved.

### Funding

The PREDICT study was funded by Health and Technology Assessment of the National Institute for Health Research, UK (grant reference 08 68 01).

### Competing interests

None of the authors have any competing interests to declare.

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