**Discordance in latent tuberculosis (TB) tests results in end stage renal disease patients.**

Jo Southern[1], Saranya Sridhar[2], Chuen-Yan Tsou[3], Susan Hopkins, Sophie Collier, Vlad Nikolayevskyy[3], Stefan Losewicz, Ajit Lalvani[2], Ibrahim Abubakar[1], Marc Lipman*

**ABSTRACT**

**Objectives:** This natural experiment was designed to assess the impact of exposure to an active case of tuberculosis in a group of immunosuppressed individual, 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 tuberculosis health care worker worked in a renal dialysis unit for a month before diagnosis, with 104 renal dialysis patients exposed for ≥8 hours.

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

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

**Conclusion:** This study suggests there is a low risk of progression to active TB in low incidence countries even in high risk groups. This plus the degree of 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.

**Highlights**

The high degree of discordance found in latent TB diagnostic tests in patients with end stage renal disease, plus the apparently low progression from latent TB infection to active disease, makes management of latent TB infection complex in this patient population.

**Key words:** Tuberculosis, ESRD, renal disease, dialysis, TST tuberculin skin test, IGRA interferon gamma release assay.

**INTRODUCTION**

Following exposure to *Mycobacterium tuberculosis* complex (M.tb), a person may develop tuberculosis (TB) regardless of their age, gender, race, religion or socioeconomic status [1]. 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, 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 [2, 3].

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 [3, 4, 5, 6], 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 cavitatory pulmonary TB, worked full time on a renal dialysis unit in a large London hospital during 2011. Due to the nature of care required in those receiving renal dialysis, the healthcare worker had prolonged close contact, of 4-5 hours on each occasion, with patients during their thrice-weekly visits. Contact would have included attaching and detaching patients from 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 participants for two years to determine disease outcome. Our objective was therefore to assess the rate of TB infection, test

conversion/ reversion, and progression from latent infection to active disease in ESRD patients exposed to a healthcare worker with pulmonary TB for a period of up to four weeks.

## METHODS

### Population

All exposed ESRD patients between 31/1/2011 and 26/2/2011 were informed of the incident and 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 interferon gamma release assay (IGRA) [Cellestis GmbH at the time of the study, now Qiagen] (QFN). Those who enrolled in the study were managed as below. All subjects were assessed for TB disease with symptom questionnaire, chest radiograph, and other tests as indicated.

### Testing regimen

All study participants had the following: blood draws for assessment using the two commercially available Interferon Gamma Release Assays (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 tuberculin skin test (TST) on enrolment, with a cut off ≥6mm for TST with no prior BCG, or ≥15mm with prior BCG as per National Institute of Health and Care Excellence (NICE) guidelines, being considered positive.

QFN was done at the Immunology Laboratory, Royal Free Hospital (London) and TSPOT at Department of Respiratory Medicine, Imperial College (London) or the National Mycobacteria Reference Laboratory of Public Health England (PHE). 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 [7, 8].

### Data collection

Information on demographics and tuberculosis risk factors as age, gender, country of birth, travel history to high TB burden countries, smoking, drug use, 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 Kruskall-Wallis tests, except for the sex 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 timepoints. Consistency over time of the IGRA is illustrated by data from those with information on every test at every timepoint.

## 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 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 male and the ethnicity of the group was diverse. Previous BCG was noted in most participants, but all were vaccinated more than five years prior to the exposure incident. The majority (69%) were non-UK born though all had lived in the UK for five years or more, and only three had visited an endemic area in recent years. Nobody was using biologics such as anti-TNF agents, though three reported taking immunosuppressive drugs. Seven (9%) had undergone a solid organ transplant. Two had had a haematological malignancy, one a jejunoileal bypass, one a gastrectomy, 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 above. All had received at least one 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 five years, and 34 (45%) currently smoked tobacco. Four reported having been homeless, three within the past five years and one was currently homeless. Nobody in the study was, or had previously been, in prison.

**Latent tuberculosis 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 ≥65 year age group: 51% overall with 77% for TST, 80% QFN and 55% TSPOT. There was higher positivity in males than females 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 ≥0.1, so no regression analyses were undertaken

**Test results at baseline, 3 months and 6 months**

Blood samples for TSPOT and QFN were collected at enrolment from 73 (97%) participants, 53 (71%) three months later and 52 (69%) at six 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 timepoint, followed by 39 at 3 months with 12 uninterpretable, and 45 at 6 months with 7 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 baseline in 14% of participants, and 17% for each of TSPOT and QFN, though not

necessarily the same individuals. The majority of participants were therefore negative for all three measures at each timepoint (tables 2 a and b).

Only 27 participants had both IGRAs at all three timepoints 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 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 baseline converted at 3 or 6 months (with one person reverting to negative). Two positive at baseline reverted.

No participant had evidence of active TB after two 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 baseline or after two years' follow up. This was surprising given their immunocompromised status and exposure to the index case over an 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 baseline, and converting to positive were similar to those observed in

UK-based older migrant populations [9]. 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 the majority of participants, the lack of progression to active 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 ESRD patients without specific TB exposure, using the definition "response <2mm to candida and/or mumps antigen", reported 40% [10] and 23% anergy [11], where a positive TST was noted in 19% and 30% respectively. A study in California found 14% TST positivity [12] – 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 [3]. Such anergy would likely by illustrated by a higher proportion of uninterpretable results compared to that seen in the general population, or an immunocompetent group. This was demonstrated when 62 ESRD patients in Switzerland were screened, where 29% were TSPOT positive and 8% indeterminate, and 8% QFN positive with 8% indeterminate [13]. It was also observed in our study, with 18% uninterpretable IGRA results according to test manufacturer specifications at study entry.

A US study in 80 renal dialysis patients tested at a single timepoint concluded "TST was not helpful in identifying LTBI in this population" [4]. They argued for the superiority of IGRAs over TST reporting a kappa correlation of 0.59 between IGRA result and conventional diagnosis, defined as a combination of chest x-ray, TST and screening questionnaire, though they found no superiority of one IGRA over the other.

Further support for IGRA compared to TST in ESRD populations was documented following routine screening of 203 such patients in Canada [6]. TST positivity was reported in 12.8% compared to 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", though 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 [14]. 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 that reported here have been documented previously in various countries. None have applied all three diagnostic measures, though similar risk factors for infection were identified. In Spain, 52 ESRD patients were exposed to a TB infected nurse for 17 weeks, and 18 (35%) were QFN positive. 3 (6%) patients were TST positive, with what they described as weak concordance – having a kappa value of 0.21 [5]. This is higher than the 16% identified in the current study, though 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 as well as induration in interpreting the TST, the kappa rose to 0.67. They concluded that QFN was more accurate in diagnosing LTBI in ESRD patients 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 ESRD patients 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 following 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.

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**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) as well as by the R&D Department of the NHS Trust involved.

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**Competing interests**

None of the authors have any competing interests to declare.

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

| | Total | *TST positive | *QFN positive | *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 |
| NK | 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 |
| NK | 20 (26) | 0 | 4 (40) | 2 (18) |
| **Time BCG given** | | | | |
| ≥5years | 37 (82) | 5 (83) | 3 (75) | 5 (55) |
| NK | 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 |

*Kruskall-Wallis tests used to compare each measure, except sex where Fisher's exact test used.

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.

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

| TST* | TSPOT* | QFN* | 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 done due to incorrect blood volume or not testable by test manufacturer instructions)** | | | |
| - | Borderline positive | Equivocal | 1 |
| + | Not done | Not done | 1 |
| - | Borderline positive | - | 2 |
| - | - | Equivocal | 2 |
| - | - | Indeterminate | 1 |
| - | + | Indeterminate | 1 |
| - | + | Not done | 1 |
| - | Not done | + | 1 |
| + | + | Not done | 1 |

*Positivity was considered ≥6mm for TST with no prior BCG or ≥15mm with prior BCG as per NICE guidelines, and as per manufacturer defined outcomes for QFT-G and TSPOT-TB

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

| | | Number of participants at |
|---|---|---|

| TSPOT | QFN | study start total = 72 n (%) | 3 months total = 46 n (%) | 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) | |

**Table 3- Results for all participants with a full set of test results i.e. valid IGRAs at each timepoint and a TST with any discordance ***

| | Study entry, time 0 | | | 3 month follow up | | 6month follow up | | consistency over time |
|---|---|---|---|---|---|---|---|---|
| SN | 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 |

*20 further participants had consistent results that were negative throughout