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Sputum host biomarkers provide accurate diagnosis of tuberculosis and may be suitable for a rapid point-of-care test http://ow.ly/tsx2S

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Serial testing using interferon-γ release assays in nursing students in India

To the Editor:

We have previously shown that Indian healthcare workers have higher prevalence of latent tuberculosis infection (LTBI) and are at increased risk for new infection [1–4]. Interferon-γ release assays (IGRAs) have been introduced as an alternative to the tuberculin skin test (TST) for diagnosing LTBI in healthcare workers and other high-risk groups. They have logistical advantages over the TST and will not cross-react with the bacille Calmette Guérin vaccine. IGRAs are now being widely used for screening healthcare workers [5], yet recent reports indicate that switching from TST to IGRAs for the serial testing of healthcare workers may result in increased rates of test conversions and reversions [3, 6–8]. Most of these studies are from low tuberculosis (TB) incidence settings, with limited opportunity for nosocomial TB exposure; as a result, the increased conversion rates are considered false-positive test conversions, making it difficult for clinicians to

interpret IGRA test conversions in these settings [9]. It remains unclear whether IGRA conversions are associated with TB exposure in high TB incidence settings where unprotected exposure to infectious TB patients is more common among healthcare workers.

To evaluate whether IGRA conversions may represent new cases of LTBI, in a high TB incidence setting, in the absence of a gold standard for LTBI, we employed TB exposure as a proxy measure. We established a cohort of nursing students who underwent IGRA testing annually for 3 years at a tertiary level hospital in southern India [1]. Annual rates of conversions and reversions were estimated in this cohort. A relationship between occupational TB exposure and change in continuous interferon-γ (IFN-γ) levels were assessed over time, in a longitudinal Tobit regression model, and association between test conversions and occupational exposure was assessed in a multivariable logistic regression model. Detailed occupational and non-occupational exposure to TB was collected through questionnaires and log books maintained by the nursing students, and this was used to track how much time each student spent on various wards. Occupational exposures assessed included total time (in months) spent working in healthcare, participation in and frequency of bronchoscopy procedures or sputum collection, days spent caring directly for pulmonary TB patients, any direct contact with pulmonary TB patients during interval between testing, days spent working on isolation wards, days spent working on pulmonary wards and days spent working in DOTS clinics.

Over the study period, 281 nursing students participated and were tested using the QuantiFERON-TB Gold In-Tube (QFT) test (Cellestis/Qiagen, Carnegie, Australia), giving a total of 674 valid QFT test results. The majority of the cohort was female (258 of 281; 92%) and the median age was 17.5 years (interquartile range 16.9–18.4 years). Most students were recruited to the study upon entry into the nursing programme and over two thirds (247 of 281; 88%) had never worked in healthcare prior to study enrolment. Among those with previous experience in healthcare or nursing, median number of weeks worked in healthcare before study enrolment was 15 weeks (interquartile range 4–24 weeks). All students had some occupational TB exposure over the 3-year study period, the mean time spent working on the isolation ward was 6.6 days per year (range 0–14 days); 44%, 30% and 23% of students reported direct contact with a smear positive pulmonary TB patient before the initial test point or during each year between testing respectively.

QFT positivity at initial testing (QFT 1) was 19.4% (54 out of 279), 22.4% (58 out of 258) at the second QFT test point (QFT 2) and 22.9% (30 out of 131) at the third (QFT 3) (table 1). Indeterminate results were rare, with only one indeterminate result at QFT 1, and two indeterminate results at QFT 3. Among those negative at initial testing (QFT 1), 9.7% (20 out of 207) had conversions at QFT 2 and a further 11.3% (11 out of 97) at QFT 3. Among those with initial positive results (QFT 1), 25.5% (13 out of 51) reverted at QFT 2 and a further 10.5% (two out of 19) reverted at QFT 3 (*i.e.* reversion after two consecutive positive results at QFT 1 and QFT 2). None of those with initial positive results who reverted at QFT 2 re-converted at QFT 3; however, among the converters at QFT 2, 72.7% (eight out of 11) experienced a reversion at QFT 3. Converters had a slightly higher median initial IFN-γ value of 0.04 (interquartile range 0.01–0.12) $IU \cdot mL^{-1}$ compared to that of 0.01 (0–0.03) $IU \cdot mL^{-1}$ in non-converters. Reverters had lower median IFN-γ values, of 0.8 (interquartile range 0.66–1.23) $IU \cdot mL^{-1}$, compared with those that did not revert, of 1.4 (0.7–5.36) $IU \cdot mL^{-1}$.

In a multivariable logistic model to assess the association between occupational and non-occupational TB exposure and QFT conversions irrespective of time, only days spent working on an isolation ward was statistically significantly associated with QFT conversions (OR 1.16, 95% CI 1.04–1.3). While the effect was small, this effect is per day and may accumulate to clinically significant levels over a nurse's career. Students with QFT conversions were offered preventive therapy and this was controlled for in both analyses, but was not statistically significantly associated with QFT reversions.

Alternative cut-off points have been suggested for the QFT test in an effort to provide improved correlation between dichotomous positive /negative QFT results and TB exposure or likelihood of LTBI. Understanding the relationship between change in continuous IFN-γ and exposure would aid in deriving appropriate cut-off points. A longitudinal Tobit regression model was used to assess the relationship between time-dependent TB exposure variables and change in continuous IFN-γ response over time, while accounting for within- and between-person variation in IFN-γ response. However, no variables (neither TB exposure nor isoniazid preventive therapy) were associated with rates of change in IFN-γ response over time in this cohort. While no students withdrew consent during the study, some students completed their nursing programme during the study and were therefore not available for repeat testing at QFT 3. All of the trajectories presented here should be interpreted as snapshots in time; while we cannot exclude the possibility that the students with missing test results at QFT 3 could have influenced study results, there is no evidence to suggest that those who were available for testing at QFT 3 would have had more or less test variability than those only available for QFT 1 and 2. This is supported by the similar rates of conversion, of

TABLE 1 Dichotomous test results over three time points

QFT 1	QFT 2	QFT 3
225 negative	187/207 negative	85/97 negative 11/97 converters (11.3%) 1 indeterminate
	20/207 converters (9.7%)	8/11 reverters (72.7%) 3/11 positive
54 positive	13/51 reverters (25.5%)	3/3 negative 0/3 converters
	38/51 positive	2/19 reverters (10.5%) 16/19 positive 1 indeterminate
1 indeterminate	1 negative	1 negative
Overall positivity rate		
19.4% (54/279)	22.4% (58/258)	22.9% (30/131)

QuantiFERON-TB Gold In-Tube test results, at initial testing (QFT 1), and second (QFT 2) and third (QFT 3) test points. One student did not complete QFT 1 but did complete testing at QFT 2 and QFT 3; this student was included in the longitudinal Tobit regression model but not in the table.

9.7% (20 of 207) at QFT 2 and 11% (11 of 100) at QFT 3, and reversion rates of 25.5% (13 of 51) at QFT 2 and 33.3% (10 of 30) at QFT 3.

In conclusion, high rates of QFT conversions and reversions were found at each test point among this cohort of young nursing students. This finding is in line with those from low TB incidence settings [8, 10]. Unlike previous studies, an association was identified between occupational TB exposure and QFT conversions (days spent working on isolation wards). This association was not robust across multiple exposure variables or analyses, despite collecting detailed occupational data and no occupational variables were associated with change in continuous IFN-γ over time.

With recent reports of unexplained conversions and reversions, concerns have arisen over the reproducibility of IGRAs when used in serial testing. There have been several studies reporting large variation in the reproducibility of IGRAs, either due to manufacturing issues [11], pre-analytical factors including phlebotomy and sample processing [12], analytical sources of variation [13, 14] and immunological sources of variation. High levels of assay variability may play an important role in explaining high rates of conversions and reversions not correlated with exposure and warrant further consideration. While the QFT test may have logistical advantages over the TST, the high rate of conversions and reversions seen in this Indian cohort suggests IGRAs may not be an appropriate tool for LTBI surveillance in this high-risk population. Ongoing efforts into novel test development should be encouraged to continue to develop improved diagnostic tests for LTBI surveillance [15].

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IGRAs are variable, yet there is an association between occupational TB exposure and conversions in Indian nurses http://ow.ly/uIbsT

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