LETTERS

Predictive value of latent tuberculosis tests in Indian healthcare workers: a cohort study

To the Editors:

Healthcare workers (HCWs) in high tuberculosis (TB)-burden countries are known to have a substantially higher risk of latent TB infection (LTBI) and TB disease [1], and this has been demonstrated repeatedly in India [2, 3]. Several studies have evaluated interferon- γ release assays (IGRAs) in HCWs [4], but no study has reported the predictive value of IGRAs in this population of HCWs at risk for TB exposure.

Between January and June 2004, we had performed a cross-sectional comparison of the tuberculin skin test (TST)

(1 tuberculin unit of purified protein derivation RT23) and QuantiFERON®-TB Gold In-Tube (QFT; Cellestis Ltd, Melbourne, Australia) in a cohort of 726 HCWs (719 had valid test results for both tests) at the Mahatma Gandhi Institute of Medical Sciences (Sevagram, India), with young trainees making up half the cohort [5]. A total of 360 (50%) HCWs were found to be positive using either the TST or QFT assay at baseline, and 226 (31%) were found to be positive using both tests. The prevalence estimates of TST and QFT positivity were comparable, with high concordance between test results [5]. Although isoniazid preventive therapy (IPT) was offered to

TABLE 1 Incidence of active tuberculosis (TB) disease according to baseline variables[#]

Baseline exposure variables	TB disease events n	Follow-up person-yr	Incidence per 1000 person-yr (95% CI)	IRR [†] (95% CI)
Age yrs				
18–20	4	1246.75	3.21 (1.20–8.55)	1.00
21–30	9	1731.25	5.20 (2.70–9.99)	1.62 (0.45–7.2)
31–40	0	432.00		
>40	1	582.08	1.72 (0.24–12.20)	0.53 (0.01–5.41)
Sex				
Males	4	1559.67	2.56 (0.96–6.83)	1.00
Females	10	2432.42	4.11 (2.21–7.64)	1.60 (0.46–7.00)
Job category				
Trainees	10	2333.75	4.28 (2.31–7.96)	1.00
Nurses	4	856.33	4.67 (1.75–12.45)	1.09 (0.24–3.77)
Physicians	0	72.00		
Others [‡]	0	730.00		
Time as HCW yrs				
1–5	11	2353.50	4.67 (2.59–8.44)	1.00
6–10	2	666.50	3.00 (0.75–12.00)	0.64 (0.06–2.94)
>10	1	972.08	1.03 (0.14–7.30)	0.22 (0.01–1.51)
TST result				
Negative [§]	8	2337.25	3.42 (1.71–6.84)	1.00
Positive [‡]	6	1654.83	3.63 (1.63–8.07)	1.05 (0.30–3.48)
QFT result				
Negative ^{##}	8	2365.75	3.38 (1.69–6.76)	1.00
Positive ^{††}	6	1626.33	3.69 (1.66–8.21)	1.09 (0.31–3.58)
TST and QFT results				
Concordant negative	7	1991.67	3.51 (1.68–7.37)	1.00
Discordant	2	719.67	2.78 (0.70–11.11)	0.79 (0.08–4.15)
Concordant positive	5	1280.75	3.90 (1.62–9.38)	1.11 (0.27–4.06)
All	14	3992.08	3.50 (2.07–5.92)	

IRR: incidence rate ratio; HCW: healthcare worker; TST: tuberculin skin test; QFT: QuantiFERON®-TB Gold In-Tube assay (Cellestis Ltd, Melbourne, Australia). [#]: n=674; [†]: none of the IRRs reached statistical significance; [‡]: includes laboratory staff and orderlies; [§]: <10 mm; [‡]: ≥10 mm; ^{##}: interferon (IFN)- γ <0.35 IU·mL⁻¹; ^{††}: IFN- γ ≥0.35 IU·mL⁻¹.

FIGURE 1. Cumulative hazard function for occurrence of active tuberculosis across tuberculin skin test (TST) and QuantiFERON®-TB Gold In-Tube (QFT; Cellestis Ltd, Melbourne, Australia) result categories (n=674). Incidence rate ratio among both TST- and QFT-positive, as compared with those with both negatives test results, at end of 2, 4 and 6 yrs is 1.54 (95% CI 0.20–11.55), 1.93 (95% CI 0.41–9.77) and 1.11 (95% CI 0.27–4.06), respectively. Cumulative hazard rate in these two subgroups is statistically similar (log-rank test p=0.84).

HCWs with positive baseline TST or QFT results, only a small proportion had completed INH therapy.

In 2010, ~6 yrs after the baseline survey, we followed-up the HCWs, using face-to-face interviews, and telephone or e-mail contact. All participants who had developed active TB disease were asked about the date of their diagnosis, nature of their TB disease and evidence of diagnosis.

We estimated the incidence rate of active TB disease (per 1,000 person-yrs) for the entire cohort, across job categories and subgroups with positive or negative TST or IGRA results, using standard definitions for a positive TST (≥ 10 mm) and QFT (interferon (IFN)- $\gamma \geq 0.35$ IU·mL⁻¹) test result. We estimated the incidence rate ratio (IRR) as the ratio of TB incidence in the exposed *versus* unexposed categories.

In 2010, we successfully contacted 674 (94%) out of 719 participants to ascertain the incidence of active TB disease. Of the 674 HCWs followed for 3,992.08 person-yrs, 14 had developed active TB disease (incidence rate 3.50, 95% CI 2.07–5.92 per 1,000 person-yrs (table 1), twice that of the Indian general population incidence of 1.70 per 1,000 person-yrs [6]). Four (28.6%) HCWs had developed pulmonary TB, while the remaining 10 had extra-pulmonary TB (four pleural, four lymph node, one abdominal and one bone/joint TB). A total of six (43%) out of 14 HCWs had evidence of smear- and/or culture-positive TB, and almost all of them were <30 yrs of age. The incidence of TB was high in young trainees, nurses and those who had worked for <5 yrs as a HCW at the time of baseline survey, although none of the IRRs reached significance (table 1).

Incidence rates of TB disease in the TST and QFT positive and negative subgroups were similar, and IRRs were close to 1.0 (null) (1.05 (95% CI 0.30–3.48) and 1.09 (95% CI 0.31–3.58) for TST and QFT, respectively). HCWs with concordant positive test results were not at a statistically significant higher risk at any

intermediate time interval during follow-up (fig. 1). HCWs who had developed active TB had similar median TST induration sizes or IFN- γ levels at baseline as those who did not progress to disease (data not shown).

In a high-burden setting, our data showed that Indian HCWs were twice as likely to develop active TB as the general population, and young trainees were particularly at risk and needed to be targeted for screening and preventive therapy. Our study also showed that a single positive QFT or TST test result at baseline, when used in isolation, did not significantly predict future risk of active TB during 6 yrs of follow-up. The predictive ability of the QFT test was similar to that of the TST, and with both tests, a large proportion of those with baseline positive results did not progress to TB disease. This is consistent with similar cohort studies from other high TB-burden countries, including South Africa [7], Gambia [8], Turkey [9], Senegal [10] and Colombia [11], which showed the comparable predictive ability of IGRA and TST. Thus, all existing LTBI tests (TST and IGRAs) may have only modest predictive value and may not identify those who are at highest risk of progression to TB disease in endemic countries.

In contrast, IGRAs appear to be more predictive in low TB-incidence settings, as shown in studies from Austria [12] and Germany [13]. In general, IGRAs appear to perform better in low *versus* high TB-incidence settings. It is likely that repeated exposures in high TB-incidence settings attenuate the predictive ability of a single cross-sectional TST or IGRA result. In addition, without performing serial testing with TST or IGRAs, it is impossible to determine the extent of exposure or whether the infection was recent (*i.e.* conversion), one of the most important factors that predict risk of progression [14].

There is growing recognition that LTBI is a spectrum, and accumulating evidence suggests that none of the existing LTBI tests can resolve this spectrum [15, 16], particularly with one-time testing [14]. It is likely that a proportion of individuals who become infected with *Mycobacterium tuberculosis* eliminate all live TB bacteria and are, thus, no longer “infected”, while they still have an adaptive immune response to the TST or IGRA, perhaps to a lesser extent with IGRAs [16]. Thus, current LTBI tests may not be able to separate out the underlying phenotypes and identify the subset that is at highest risk of future disease, as confirmed by a new meta-analysis [17]. Therefore, the search for more predictive biomarkers or combinations of biomarkers and risk factors must continue.

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Microsatellite alterations at 3p and 19q in EBC DNA of smokers: are they reversible after smoking cessation?

To the Editors:

Carcinogens in cigarette smoke may leave fingerprints in the bronchial tissue in the form of specific mutations that initiate cancer development [1]. Numerous genetic alterations have been recognised as critical effects of cigarette smoke and studied in the airways of current and former smokers. Microsatellite alterations (MAs) at 3p and 19q are among the most studied, being considered useful markers of genetic susceptibility and genome destabilisation in susceptible smokers [2, 3]. Instability and/or loss of heterozygosity at 3p and 19q have largely been reported in the lung tissue, sputum and blood of smokers and lung tumour patients [2, 4]. These mutations, which are considered an early effect of cigarette smoke, are dose-dependent and related to the number of cigarettes smoked in a lifetime [3]. In addition, short-term exposure to cigarette smoke seems to cause MAs at 3p that are not necessarily a consequence of the development of a neoplastic mass [5].

Our group recently reported MAs at these loci in the exhaled breath condensate (EBC) of smokers and patients affected by

nonsmall cell lung cancer, demonstrating that EBC could be a surrogate for tissue in assessing tobacco-induced molecular damage in the lungs [2, 3].

Interestingly, although smoking cessation may reverse the clonal expansion of abnormal cells, the persistence of some genetic alterations in former smokers indicates that a high rate of clonal genetic damage persists even after smoking cessation [6].

Several genetic susceptibility markers have been studied after smoking cessation and classified as rapidly reversible, slowly reversible or irreversible [7]. Indeed, to know whether a gene alteration is reversible could turn out to be important, as rapidly reversible genes seem to have different biological functions than slowly reversible or irreversible genes [7].

The aim of this study was to test the reversibility of MAs at 3p and 19q by analysing these markers for the first time in the EBC and whole blood (WB) of smokers at baseline (T0) and then at 12 months after smoking cessation (T1). The correlations between MAs with sex, pack-yr, exhaled carbon monoxide and Fagerström nicotine dependence score were also investigated.