USE OF INTERFERON-γ RELEASE ASSAYS (IGRAs) IN TUBERCULOSIS CONTROL

IN LOW- AND MIDDLE-INCOME SETTINGS

EXPERT GROUP MEETING REPORT
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Executive summary

Background

Research over the past decade has resulted in the development of two commercial interferon-gamma release assays (IGRAs). Both assays work on the principle that the T-cells of an individual who have acquired TB infection will respond to re-stimulation with *M. tuberculosis*-specific antigens by secreting interferon-gamma. The QuantiFERON-TB Gold (QFT-G, Cellestis, Australia) and the newer generation QuantiFERON-TB Gold In-Tube (QFT-GIT, Cellestis, Australia) are whole-blood based enzyme-linked immunosorbent assays (ELISA) measuring the amount of IFN-γ produced in response to three *M. tuberculosis* antigens (QFT-G: ESAT-6 and CFP-10; QFT-GIT: ESAT-6, CFP-10 and TB7.7). In contrast, the enzyme-linked immunospot (ELISPOT)-based T-SPOT.*TB* (Oxford Immunotec) measures the number of peripheral mononuclear cells that produce INF-γ after stimulation with ESAT-6 and CFP-10.

In recent years, IGRAs have become widely endorsed in high-income countries for diagnosis of latent TB infection (LTBI) and several guidelines (albeit equivocal) on their use have been issued. Currently, there are no guidelines for their use in high TB- and HIV-burden settings, typically found in low-and middle-income countries, where IGRA use are being marketed and promoted, especially in the private sector. Systematic reviews have suggested that IGRA performance differs in high- versus low TB and HIV incidence settings, with relatively lower sensitivity in high-burden settings. The majority of IGRA studies have been performed in high-income countries and mere extrapolation to low- and middle-income settings with high background TB infection rates is not appropriate. The WHO Stop TB Department has therefore commissioned systematic reviews on the use of IGRAs **in low- and middle-income settings**, in pre-defined target groups, with funding support from the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and TREAT-TB/The Union. The target groups and major findings are briefly summarised below.

Summary of results

Use of IGRAs in diagnosis of active TB: IGRAs were explicitly designed to replace the TST in diagnosis of LTBI, and were not intended for diagnosis of active TB. Because IGRAs (like the TST) cannot distinguish LTBI from active TB, these tests are expected to have poor specificity for active TB in high-burden settings due to a high background prevalence of LTBI. Nineteen studies simultaneously estimating sensitivity and specificity among 2,067 TB suspects demonstrated a pooled sensitivity of 83% (95% CI 70% - 91%) and pooled specificity of 58% (95% CI 42% - 73%) for T-SPOT (8 studies), and a pooled sensitivity of 73% (95% CI 61% - 82%) and pooled specificity of 49% (95% CI 40% - 58%) for QFT-GIT (11 studies). There was no consistent evidence that either IGRA was more sensitive than the TST for diagnosis of active TB diagnosis. Two studies evaluated the incremental value of IGRAs and found no meaningful contribution of IGRAs for diagnosis of active TB beyond readily available patient data and conventional microbiological tests.
Expert Group consensus: *The quality of evidence for use of IGRAS in diagnosis of active TB was low and it is recommended that these tests should not be used as a replacement for conventional microbiological diagnosis of pulmonary and extra-pulmonary TB in low- and middle-income countries (strong recommendation). The Expert Group also noted that current evidence did not support the use of IGRAs as part of the diagnostic workup of adults suspected of active TB in low-and middle-income countries, irrespective of HIV status. This recommendation places a high value on avoiding the consequences of unnecessary treatment (high false-positives) given the low specificity of IGRAs in these settings.*

Use of IGRAs in children: Only two small studies were identified which prospectively estimated incidence of active TB in children who had been tested with QFT. Conflicting results were reported. When the reference standard for LTBI was exposure, all three tests (TST, QFT and T-SPOT) seemed to be associated with the

level of exposure (categorised either dichotomously or by an exposure gradient); however, methodological inconsistencies between the studies regarding the selection and definition of reference standards for active TB and exposure limited the comparability of studies and results. Estimates of association were very similar, suggesting no difference in performance between TST and IGRAs for diagnosis of LTBI and active TB in children.

Expert Group consensus: *The quality of evidence for use of IGRAS in children was very low and it is recommended that these tests should not be used as an alternative to TST in paediatric TB in low and middle-income countries for the diagnosis of latent TB infection, or as an alternative to TST in the workup of a diagnosis of active TB disease in children, irrespective of HIV status (strong recommendation). The Expert Group also notes that there may be additional harms associated with blood collection in children and that issues such as acceptability and cost have not been adequately addressed in any studies.*

Use of IGRAs in HIV-infected individuals: 36 studies were identified that included 5,400 HIV-infected individuals. In persons with active TB (used as a surrogate reference standard for LTBI), pooled sensitivity estimates were higher for TSPOT (72%, 95% CI 62% - 81%, 8 studies) than for QFT-GIT (61%, 95% CI 41% - 75%, 8 studies). Large prospective cohort studies have established that persons with a positive TST have a 1.4 to 1.7-fold higher rate of active TB within one year compared to persons with a negative TST result. Three studies evaluating the predictive value of IGRAs in HIV-infected individuals showed that IGRAs have poor positive predictive value but high negative predictive value for active TB. While these results suggest that a negative IGRA result is reassuring (no person with a negative IGRA result developed culture-positive TB), the studies had serious limitations, including small sample sizes with short-duration of follow-up and differential evaluation and/or follow-up of persons with positive and negative IGRA results. Neither IGRA was consistently more sensitive than TST in head-to-head comparisons, and the impact of advance immunosuppression on IGRA validity remains unclear: Two studies reported TST and IGRA data stratified by CD4 count. In one study, the proportion of positive results among those with CD4 cell count <200 decreased by 27% (95% CI -61, 8) with TSPOT and 35% (95% CI -59, -11) with TST. In the other study, the proportion of positive results among those with CD4 cell count <200 decreased by 31% (95% CI (-53, -9) with TSPOT and increased by 15% (95% CI (-11, 41) with TST. All tests therefore seem to be affected by CD4+ cell count, and additional studies from low/middle income countries are needed.

Expert Group consensus: T*he quality of evidence for use of IGRAS in individuals living with HIV infection was very low and recommended that these tests should not be used as a replacement for TST for the assessment of LTBI (strong recommendation). This recommendation also applies to HIV-positive children based on the generalisation of data from adults.*

Use of IGRAs in health care worker (HCW) screening: Limited data was available on the utility of screening HCWs for LTBI in high incidence countries. Three cross-sectional studies were evaluated comparing IGRA and TST performance in HCWs in three countries, although TST was only performed in two of these. TST and IGRA positivity rates were high in HCWs, ranging from 40% to 66%. IGRA positivity was slightly lower than TST positivity in the two studies comparing TST and IGRAs; however, the difference in estimated prevalence was significant in one study only. Serial testing data, evidence on the predictive value of IGRAs in HCWs, as well as reproducibility data are still absent for high-incidence settings and limited even in low-incidence settings.

Expert Group consensus: T*he quality of evidence for use of IGRAS for screening of health care workers in low- and middle-income countries was very low and it is recommended that these tests should not be used in health care worker screening programmes (strong recommendation). The Expert Group also noted the lack of WHO policy on using the TST in health care worker screening programmes.*

Use of IGRAs in contact screening and outbreak investigations: 16 studies (14 original manuscripts and 2 unpublished studies) were identified which evaluated IGRAs in contact screening and outbreak investigations in low- and middle income countries. Seventy-five percent (12/16) of contact studies included children in their study populations. The majority of studies were cross-sectional and looked at concordance between TST and IGRAs. Due to significant heterogeneity in study designs and outcomes

assessed in each study, it was not possible to pool the data. The majority of studies showed comparable LTBI prevalence by TST or IGRA in contacts and only 4 studies reported a statistically significant difference between positivity rates estimated by TST, SPOT.TB or QFT. The most commonly observed discordance was of the TST-positive/IGRA-negative type. Both IGRAs and the TST seemed to show positive associations with higher levels of exposure in cross-sectional studies, but the strength of the association (ie. adjusted odds ratio) varied across studies. Results indicated that concordance between TST and IGRAs ranged widely, with only moderate agreement. In high-income settings, IGRAs appear to be dynamic and are associated with conversions and reversions which has impact for serial testing of contacts; however no data exists for LMICs.

Expert Group consensus: The quality of evidence for use of IGRAS for LTBI screening in contact and outbreak investigations was very low and it is recommended that these tests should not be used as a replacement for TST, neither in adults nor children investigated as close contacts of patients with confirmed active TB (strong recommendation).

Predictive value of IGRAs: Three studies provided incidence rate ratios (IRR) of TB stratified by IGRA as well as TST status at baseline. The association with subsequent incident TB in test-positive individuals compared to test-negatives appeared higher for IGRA than for TST; however, this was not statistically significant (IGRA: IRR=3.24; 95CI 0.62-5.85; I^2=0%; p=0.90; TST: IRR=2.28; 95CI 0.83-3.73); The Expert Group also noted that both IGRAs and TST seemed to show positive associations between exposure gradient and test results but with variability in the strength of the association across populations irrespective of BCG vaccination. No statistically significant increase in incidence rates of TB in IGRA- positives compared to IGRA-negatives was observed and the vast majority of individuals (>95%) with a positive IGRA result did not progress to active TB disease during follow-up. Both IGRAs and the TST appeared to have only modest predictive value and did not help identify those who are at highest risk of progression to disease. The predictive value for serial testing could not be assessed as all three studies performed single time-point IGRA testing. Patient relevant outcomes based on sensitivity and specificity appeared comparable between IGRAs and the TST.

Expert Group consensus: *The quality of evidence for the predictive value of IGRAS was very low and it is recommended that these assays should not be used to identify individuals at risk of active TB disease in low- and middle-income countries (strong recommendation).*

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