What is the clinical utility of interferon-γ release assays for the diagnosis of TB in high-TB-burden countries?

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The advent of interferon-γ release assays (IGRAs) has provided a new insight into the diagnosis and immunology of TB. IGRAs are being widely accepted and marketed in low-TB-burden countries. However, globally, the impact of TB is concentrated in 22 high-burden countries, where high percentages of the population are infected with TB, HIV coinfection rates are high, continuous community TB exposure occurs, laboratory infrastructure is weak and latent TB infection is not treated. What role do these new assays play in these settings? Can they really have an important benefit in these countries, among people who most need an innovation in TB diagnostics? Here, we examine the most current literature in order to answer specific diagnostic questions that are relevant to these settings.

The global TB case notification rate has fallen short of the 70% case-detection target set for 2005 [1], reinforcing the urgent need for development and implementation of novel and improved diagnostic tests for TB. The application of novel technologies for TB diagnosis in high-TB-burden countries (HBCs) has been defined as a research priority by the WHO [1]. The technical limitations of the conventional diagnostic test (sputum smear microscopy), including the associated cost of delayed diagnosis, has been recently reviewed [2].

The tuberculin skin test (TST) is the traditional method used for the diagnosis of *Mycobacterium tuberculosis* infection. The TST contains a mixture of a large number of antigens common to many *Mycobacterium* species and is, therefore, confounded to varying degrees by the effect of *Bacillus* Calmette–Guerin (BCG) vaccine [3]. Nontuberculous mycobacterial (NTM) infections, increasingly described in HBCs, may also cross-react with TST, causing false-positive reactions.

In the last 3 years, there has been an explosion in interest in the diagnostic implications of interferon (IFN)-γ release assays (IGRAs) for the detection of TB. IGRAs are *in vitro* blood tests that measure cell-mediated immunity to TB by detecting IFN-γ released by T cells after exposure to antigens, such as early secreted antigen target (ESAT)-6 and culture filtrate protein (CFP)-10. These antigens are not present in BCG or most NTM infections, making IGRAs more specific than TST [2,4].

Two IGRAs are now commercially available. QuantiFERON®-TB Gold in-tube assay (QFT) (Cellestis, Victoria, Australia) is a whole-blood ELISA that measures IFN-γ response to antigens ESAT-6, CFP-10 and TB7.7. The T-SPOT.TB Assay (ELISPOT) (Oxford Immunotec, Oxford, UK) detects the number of T cells that produce IFN-γ in response to ESAT-6 and CFP-10 [2]. IGRAs contain positive (mitogen) and negative (no antigen) controls, which are used to assist in the interpretation of patient results. Several in-house assays are also being used as research tools, but are not standardized enough for widespread clinical application.

The potential advantage of a single-visit blood test in a HBC, where TB patients often do not return for follow-up visits, is clear; however, the IGRA does require a complex system of laboratory infrastructure, including trained staff, refrigeration and reliable electrical power, which may not be available in a HBC setting.

Most literature, to date, describes the use of IGRAs in low-TB-burden countries, for the diagnosis of latent TB infection (LTBI), in order to guide isoniazid prophylaxis and trace contacts of active TB. In this setting, they have been shown in systematic reviews to be more specific than TST, to correlate better with markers of exposure to TB and have less cross-reactivity with BCG vaccination and NTM infection [3,5]. Guidelines in these countries have suggested that they can be used to replace TSTs in all clinical situations [6].

The global burden of TB is, however, disproportionately borne by 22 HBCs [7]. Over 80% of new TB patients in 2004 were living in African, south-east Asian and western Pacific countries [7]. Most of these countries do not have widespread access to quality-controlled mycobacterial culture, limiting their capability to make an accurate diagnosis of active TB [2].

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High-TB-burden countries have distinct socioepidemiological differences from low-burden countries, which may influence the feasibility and performance of IGRAs. Generally, HBCs have a high (30–50%) prevalence of LTBI, widespread BCG coverage and variable and unmeasured NTM infection rates. Selected HBCs have critically high HIV seroprevalence rates. In addition, malnutrition and tropical infections, such as helminthiasis, have the potential to affect the performance of immune-based assays by altering immunological capacity and priming.

The detection of active, infectious TB cases is the highest priority for TB control programs in HBCs. Although the treatment of LTBI with isoniazid alone (also called prophylactic treatment) may result in a reduction in the risk of active disease and increased life expectancy in a single patient [8], this intervention may not benefit the community in a HBC. Cost, feasibility, risk of erroneously prophylaxing patients with active TB, and drug toxicity limit the suitability of widespread prophylaxis. HBCs can not prioritize this intervention when ongoing active disease transmission has not been controlled, except in high-risk groups such as young children and HIV-infected hosts [8]. Until a national program can provide a 90% treatment completion rate, LTBI treatment is not feasible [9].

The search strategy and results have been taken with permission from a previous publication [5]. Briefly, a comprehensive database of articles on IGRAs has been developed and updated using the following PubMed search string: ([interferon-γ release assay*] OR [T-cell-based assay*] OR [antigen-specific T cell*] OR [T cell response*] OR [T-cell response*] OR [interferon*] OR [interferon-gamma] OR [gamma-interferon] OR [IFN] OR [elispot] OR [ESAT-6] OR [CFP-10] OR [culture filtrate protein] OR [Enzyme Linked Immunosorbent Spot] OR [Quantiferon* OR Quantiferon-TB]) AND ([tuberculosis OR mycobacterium tuberculosis]). The search was limited to human studies published in English. Additionally, a hand search of all issues of the *International Journal of Tuberculosis and Lung Disease* that were published over the past 5 years was carried out.

From this database of articles, we hand-selected those articles that met both of the following inclusion criteria:

- Studies took place in a HBC
- Studies reported on four predefined diagnostic questions relevant to HBC

We have defined four clinical diagnostic categories in which IGRAs may offer advantages useful enough to justify their increased cost and to replace conventional tests in HBC. These four questions are clinical applications in which the role of IGRA is not clear:

- Diagnosis of active TB among adults, especially among HIV-infected patients
- Management of children suspected to have active disease and those who are contacts of parents with active TB
- Prediction of onset of active TB disease
- Prediction of outcome of TB therapy

Performance for the diagnosis of active TB among HIV-positive adults in high-TB-burden countries

Of essential importance to the application of IGRAs in HBCs is the performance among hosts compromised by HIV. Patients unable to mount an adequate T-cell response to a mitogen stimulus found in the positive control tube may give an indeterminate IGRA result (positive-control failure). Indeterminate results do not represent that the blood does not recognize TB antigens (IGRA negative), but that it can not mount a cell-mediated immune response at all. The clinical interpretation of indeterminate IGRA results is not yet well defined, but an indeterminate result is more clinically useful than a false-negative result, which would erroneously suggest that the diagnosis of TB could be ruled out. Ideally, IGRA interpretation should be correlated with immunological status, but $CD4^+$ cell-count testing may not be available to all patients with HIV in HBCs.

HIV infection itself may reduce IFN-γ response. In asymptomatic Ugandan adults, 22 with HIV and 75 without HIV, IFN-γ response to TB antigens purified tuberculin antigen (PPD) and CFP was significantly weaker among HIV-positive patients ($p < 0.001$) [10]. HIV infection causes a shift from Type 1 immune response to Type 2, and, therefore, production of IFN-γ is downregulated. This could partly explain indeterminate IGRA results in HIV-infected individuals [11].

Three groups of patients were sequentially enrolled from one HIV testing center in Khayelitsha township, Cape Town, South Africa, an area with a local incidence of active TB of 1612/100,000. A total of 41 patients had HIV and culture- or smear-positive active pulmonary TB, 41 were HIV-positive without symptoms or signs of active TB and 41 were HIV-negative

controls. All were assessed with TST and in-house IFN-γ and ELISPOT assays to various TB antigens. Response to ESAT-6, CFP-10 and PPD was higher in the HIV with active TB group compared with the HIV without TB group ($p < 0.04$), despite the fact that the first group had significantly lower $CD4^+$ cell counts. Among the asymptomatic groups, HIV decreased the TST and IFN-γ responses to PPD ($p < 0.001$), but the ELISPOT responses to ESAT-6, CFP-10, TB10.3 and Acr2 were not significantly decreased. The overall sensitivity of IGRA for active TB among HIV-infected patients was 81–90%.

This study suggested that a ratio of ELISPOT count divided by the $CD4^+$ cell count of more than 1 had 88% sensitivity and 80% specificity for the diagnosis of active pulmonary TB in HIV-infected individuals. This ratio could, therefore, be interpreted to suggest active TB in the HIV-infected host. However, if the ratio is less than 1, active disease may not be reliably excluded but, if other tests for active disease are negative, the patient could be safely treated for LTBI only [12]. This well-designed study suggests that in-house ELISPOT response to certain TB antigens is not compromised by HIV, and that among HIV-infected individuals, ELISPOT may have some predictive capacity for active TB. To date, this type of ratio has not been described using commercial IGRA tests.

A total of 154 adults with culture-confirmed active TB (143 pulmonary, seven pleural and four other) were consecutively studied in Cape Town, South Africa. QFT was performed a median of 3 days after treatment began. A total of 23 patients (15%) had indeterminate results, but among the other 131, sensitivity for active TB was 76% (95% CI: 68–83%). Only 41 patients underwent HIV testing. QFT was more sensitive for active TB among HIV-infected (81% [60–92]) than HIV-uninfected patients (73% [48–89]). The $CD4^+$ correlation was not reported. HIV was associated with a significantly lower IFN-γ response ($p = 0.033$). TST sensitivity among the 146 patients who had a TST result was 90% (84–94%), and 85% among HIV-infected patients (induration cutoff >5 mm) [13]. In conclusion, TST demonstrated a trend to greater sensitivity for active TB among HIV-infected patients compared with IGRA.

A total of 50 Zambian adults with smear-positive pulmonary TB were tested with ELISPOT [14]. Out of 39 (90%) HIV-positive TB cases, 35 were detected by ELISPOT to ESAT-6 or CFP-10, while 11 out of 11 HIV-negative TB cases were detected ($p = 0.52$). $CD4^+$ cell counts were not available. There was no difference in counts of IFN-γ-producing cells between HIV-positive and -negative subjects. Thus, sensitivity of ELISPOT for active pulmonary TB among HIV-infected patients was high, and the magnitude of response did not appear to be compromised by HIV.

Three clinical studies among HIV-infected patients produced similar estimates of sensitivity of IGRAs for active TB of 81–90%. Two studies suggest that HIV does not reduce response to TB antigens, but one study suggests that it does. It is unclear whether IGRAs are less [12] or more [13] impaired than TST by the presence of HIV.

The high rate of background LTBI among HIV-infected patients in HBCs will continue to impair the specificity of IGRAs. If $CD4^+$ counts are available, the ratio of (in-house) IGRA response to $CD4^+$ count could outperform smear among HIV patients with active TB, but this performance advantage will still not justify the large cost difference between IGRAs and smears among HIV patients.

Performance for the diagnosis of active TB in HIV-negative adults in high-TB-burden countries

In HBCs, where LTBI is common, a positive IGRA result cannot distinguish active TB from LTBI. This limits the utility of the test to those patients with negative results. It has been suggested, however, that quantitative IFN-γ expression among HIV-negative patients might be more useful than a positive or negative response.

In a district hospital in Rio de Janiero, Brazil, 60 HIV-negative adults with smear- or culture-positive active TB (50 pulmonary and ten pleural) were compared with a control group of 20 healthy volunteers, leprosy patients and patients with pulmonary disease other than TB. The percentage of positive IGRA results in the study and control group was similar, but the level of IFN-γ released (as detected by in-house ELISA) in response to ESAT-6 was significantly higher in the patients compared with the controls [15]. There was also a significant difference between the IFN-γ response in pleural TB patients and cavitary pulmonary TB patients compared with that of controls ($p = 0.03$ and $p = 0.02$, respectively), whereas noncavitary patients demonstrated no difference ($p = 0.20$).

This is the only study that has addressed this question, to date, in a HBC. However, one Swiss study has also demonstrated that quantitative

IGRAs trended higher in culture-positive TB cases than TB contacts, and that a certain cutoff could distinguish LTBI from active TB with 83% sensitivity and 74% specificity [16].

Therefore, early evidence suggests that quantification of IFN-γ may help distinguish active TB from LTBI and increase specificity for active TB, especially among cavitary pulmonary TB. IGRA manufacturers have defined cut-off values for positivity of commercial assays based on optimization of test sensitivity for the detection of LTBI in low-burden countries. This fact may contribute to higher false-positive rates when applied for active TB in HBCs, because the prevalence of LTBI is high and continuous TB exposure may prime the immune system. Cut-offs for new diagnostic tests are often defined by receiver operator characteristic (ROC) curve analysis. In HBC, ROC curves for these tests need to be defined by comparing microbiologically confirmed active TB cases with cases of LTBI [11].

Among HIV-negative adults in HBCs, currently available commercial IGRAs do not have adequate specificity for use in the diagnosis of active TB.

Performance for diagnosis among children in high-TB-burden countries

TB among children is recognized as a significant contribution to the global TB burden [17]. Children below the age of 5 years have an increased risk of progression to active TB following exposure, and should be screened and offered prophylactic treatment [18]. Symptomatic children should be investigated for active TB and asymptomatic children should be risk stratified [18].

Performance for diagnosis among symptomatic children suspected to have active TB

In KwaZulu-Natal, South Africa, 293 children with suspected active pulmonary and extrapulmonary TB were investigated with ELISPOT and conventional methods [19]. Among 133 with confirmed or highly probable TB, ELISPOT was positive in 83% (75–89%) and TST was positive in 63% (54–72%). Four of 13 (31%) in whom active TB was ruled out also had positive ELISPOT.

Among these children, ELISPOT would have supported a diagnosis of TB in 11 out of 21 (52%) smear-negative, culture-positive patients, offering microbiological diagnosis and earlier treatment as compared with waiting for culture results. In subgroups including 30 patients with HIV, ELISPOT was 73% sensitive (54–88%), while TST was 36% sensitive (18–58%), and among 59 very malnourished children (weight-for-age Z scores below norm by 2 standard deviations), ELISPOT sensitivity was 78% (65–88%) and TST sensitivity was 44% (30–59%).

Another South African study of 70 HIV-negative children started on anti-TB therapy for suspected TB measured ELISPOT response to ESAT-6 or CFP-10 [20]. A positive response to any antigen was found among ten out of 12 (83%) with culture-confirmed TB, 34 out of 47 (72%) with probable TB and five out of 11 (45%) with possible TB ($p = 0.05$, comparing culture positives with all clinically diagnosed TB). Overall, detectable responses were observed in 70% of the children. The weight-for-age Z score did not correlate with any test response.

In a study in India, 24 HIV-negative children with culture proven or clinically suspected TB (22 with pulmonary TB, one with TB meningitis and one with military TB) were compared with 22 HIV-negative children who were positive tuberculin reactors (mean TST 16.2 ± 3.3 mm). IFN-γ concentrations in *M. tuberculosis* stimulated peripheral blood monocytes were higher for healthy tuberculin reactors than for patients with tuberculosis ($p = 0.02$). IFN-γ production was most severely depressed in patients with moderately advanced and far-advanced pulmonary disease and in malnourished patients [21]. This would suggest that active TB itself may depress IFN-γ production.

Among symptomatic children with suspected active TB, IGRAs offer a sensitivity advantage over TST, especially among children with risk factors for a false-negative TST, such as malnutrition or HIV. Symptomatic children have a lower likelihood of LTBI than symptomatic adults in HBCs, which may improve the specificity of IGRA among children as compared with adults. In addition, IGRAs may be beneficial among symptomatic children because of the difficulty in obtaining diagnostic specimens from children, the tendency to extrapulmonary TB presentation and because of the rapid results achieved as compared with cultures.

Performance among children who are asymptomatic contacts of active TB

Children who are in direct contact with adults with active TB represent a difficult diagnostic challenge. Despite the lack of symptoms, young contacts (<3 years) are at high risk of progression to active TB, and HIV-infected children remain at

high risk at older ages as well. The triad of TB contact, evidence of LTBI and abnormal chest x-ray may not perform adequately for the diagnosis of active TB among children in HBCs, since exposure may be continuous and undocumented [22].

Among 207 children in Nigeria, three groups were defined: contacts of adults with smear-positive TB, contacts of adults with smear-negative TB, and controls. TST was greater than 10 mm in 38 out of 78 (49%), 13 out of 83 (16%) and six out of 46 (13%) in the three groups, respectively, and QFT was positive in 53 out of 72 (74%), eight out of 81 (10%) and four out of 39 (10.3%) ($p < 0.01$). This demonstrates that the QFT may have some discriminatory capacity among these groups, with contacts of smear-positive adults providing a higher positivity rate. Compared with QFT, TST may underestimate the risk of LTBI among children [23].

A clinically relevant question among children in HBCs is the influence of the BCG vaccine on IGRAs and TSTs. A total of 105 consecutively admitted children (82% of whom had BCG scars) with suspected TB or a history of contact with active TB were recruited at a rural hospital in India. BCG was not associated with the results of either TST or QFT ($p > 0.05$ for both tests). Agreement between TST and QFT results was 100% ($\kappa = 1.0$) in BCG scar-negative children as compared with 94% ($\kappa = 0.63$) in scar-positive children [24].

The contribution of IGRAs to the diagnosis of asymptomatic children is still unknown. Like TST, they are not able to distinguish active TB from LTBI in a child without radiological abnormalities [25]. The potential future applications of IGRAs may be found among high-risk HIV-infected children or as a 'rule-out' test for active TB [22]. IGRAs have not demonstrated consistent enough advantages at this point to replace the TST in this group.

Performance for prediction of onset of active TB in high-TB-burden countries

If the IGRA response can be used to prospectively predict the development of active TB among those with LTBI, it may be useful in selected high-risk groups to offer prognostic information or guide prophylactic treatment.

A cohort of 631 HIV-infected Ugandans at high risk for active TB was followed over a median period of 1.5 years, and quantitative ELISA was performed to measure the cytokine responses to mycobacterial antigens PPD and CFP. There was a strong association between IL-2 response to mycobacterial antigens and risk of progression to active TB (adjusted relative risk: PPD: 3.48; CFP: 3.99; $p < 0.001$), but IFN-γ responses showed no such association [26].

A total of 24 healthy Ethiopian TB contacts were followed up at 2 years after recruitment [27]. A total of 12 developed symptoms suggestive of active TB over the time period, seven of which were confirmed as active TB. The mean baseline IFN-γ production to ESAT-6 stimulation was higher among those who subsequently developed active TB than among those who developed other diseases or healthy contacts ($p < 0.001$), whereas TST response had no such association.

Preliminary studies would suggest that baseline IGRA values may have predictive capability, but further work among TB contacts sampled regularly over long periods will be needed in order to determine if regular IGRA testing will be able to divide patients into low- and high-risk groups.

Performance for prediction of outcome of therapy for active TB in high-TB-burden countries

IGRAs may prove to be useful adjuncts or biomarkers for monitoring the efficacy of anti-TB therapy. No single test can replace the conventional clinical, microbiological and radiological parameters that are used to monitor response to treatment [28], but additional information may give an indication of treatment failure earlier than smear persistence, suggesting the need for drug-susceptibility testing. Decrease in IGRA response after successful treatment could also help to individualize the duration of therapy, especially in patients with severe or cavitary disease, or among those with HIV infection who are at risk of reinfection. This could prove useful as a biomarker for the assessment of new pharmacological or immunological interventions for treating multidrug-resistant (MDR) TB, for which the response to treatment and the early bactericidal activity may be imperfect surrogate markers [28].

A total of 89 Gambian adults with smear- and culture-positive TB were tested by ELISPOT at diagnosis and after 12 months of treatment [29]. In total, 60 out of 82 patients who successfully completed therapy had a significant decline in ELISPOT to CFP-10, and 64 out of 82 had a significant decline in ELISPOT to ESAT-6 ($p < 0.001$ for both).

In 60 microbiologically proven active pulmonary TB patients in India, a QFT assay was conducted at baseline, at 2 months and then at

6 months. At baseline, 73% of patients had a positive QFT; at 2 months, 81% were positive; and at 6 months, 79% were positive. Although there was a slight but variable decrease in INF-γ levels over time, no clear correlation between antigen burden and T-cell responses were observed [30].

A small subset of ten children with suspected active TB within a diagnostic study from South Africa had 1-, 3- and 6-month follow-up ELISPOT to ESAT-6, CFP-10 and PPD during treatment [20]. An initial increase in spot-forming cells was observed ($p = 0.004$), followed by a significant decrease at 3 and 6 months for all three assays.

A total of 18 Africans with active pulmonary TB were sampled before and after treatment, although time intervals were not reported. Significant increases in IFN-γ production to ESAT-6 and PPD were observed [31].

There are a few available studies from HBCs prospectively analyzing IGRA response after prophylactic treatment for LTBI. The immunological response in this group may not be directly comparable with that of active TB patients under treatment. Healthcare workers who accepted preventive therapy for LTBI in India were serially assessed at 4 and 10 months following the completion of 6 months of isoniazid treatment [32]. Ten were available, nine of whom had elevated IGRA at baseline. There was a nonsignificant decline in quantitative IFN-γ expression to ESAT-6, CFP-10 and TB7.7 antigens.

Among 33 cases of LTBI who were administered isoniazid preventative therapy in South Africa, a 1.8-fold increase in IFN-γ-producing cells was observed after 26 ± 4 days of treatment ($p = 0.006$) [33]. At the end of 82 ± 6 days, however, a significant decrease in cells was observed ($p = 0.04$). Cases who declined medical treatment had no change in cell counts.

On balance, IGRAs do not have a reliable prognostic capability for TB patients undergoing treatment, demonstrating widely variable responses under treatment. There are several hypotheses to explain the absence of a good correlation between successful treatment and reduction in IFN-γ expression. Ongoing TB exposure with continued antigenic stimulation, inadequate duration of follow-up, use of QFT rather than more sensitive ELISPOT, and variability in the reproducibility of the test may contribute to this confounding [30]. An initial increase in IFN-γ expression, followed by a decrease observed after successful treatment, would suggest that T-cell immunity is activated during therapy and then decreases with successful treatment, which would parallel the clinical understanding of drug effect. However, this has not yet been reliably observed, and further large trials of patients in HBCs under active TB treatment are needed.

Serial testing with IGRAs may be problematic for several reasons. The immunological understanding of test conversions and reversions over time is incomplete.

The quantitative relationship between levels of effector T cells, antigen load and bacterial burden is not fully understood [28], and IGRAs may be influenced by considerable inter- and intra-individual variations over time. Further prospective studies are needed to assess the clinical and immunological implications of conversions and reversions during treatment of active and latent TB [34].

Conclusion

There is considerable interest in the application of IGRAs, and they may offer a new window of insight on the nature and behavior of TB infection and host response [5]. However, most research investment has been in low-burden countries, where active TB incidence is low and LTBI diagnosis and treatment are relevant.

Current IGRAs do not offer an advantage over TST for the diagnosis of active TB among HIV-infected adults in HBCs, where high LTBI rates will compromise specificity. In a ratio with available $CD4^+$ counts, however, IGRA may offer a performance advantage over TST. Among HIV-negative adults, quantitative in-house IGRA may help distinguish LTBI from active TB.

Symptomatic children with suspected active TB may benefit from the increased sensitivity of IGRAs over TST, especially when malnourished or HIV infected. Asymptomatic children who are contacts of adults with active TB will not benefit from IGRAs compared with TSTs.

The data on prediction of onset of active TB or outcome of TB treatment are very preliminary. Until a better understanding of test conversions and reversions is achieved, this application will not be clinically useful. There is a paucity of good-quality prospective evaluations performed in HBCs, and this should be a priority for further research and funding.

The limitations of IGRAs include the uncertain interpretation of indeterminate results, laboratory complexity, and cost.

Executive summary

- Interferon-γ release assays (IGRAs) measure cell-mediated immune response to TB antigens.
- Research into IGRAs for TB diagnosis has focused on low-TB-burden countries, where TB exposure is rare and latent TB infection (LTBI) is treated.
- IGRAs may not be a useful tool for the diagnosis of TB infection in high-burden countries (HBCs) owing to the cost, complexity, low specificity and indeterminate results.
- Four clinical diagnostic categories that may justify IGRA use in HBCs include detection of active TB among HIV-positive and -negative adults, detection of active TB among symptomatic children and managing children exposed to adults with active TB, prediction of onset of active TB, and monitoring of the effectiveness of TB treatment.

Performance for the diagnosis of active TB among HIV-positive adults in high-burden countries

- IGRA sensitivity among HIV-infected patients with proven TB is 81–90%.
- Some evidence suggests that HIV impairs IFN-γ production.
- Indeterminate results occur when the host can not mount an adequate mitogen response, thereby leading to a failure of positive controls.
- The interpretation of indeterminate results is not yet well defined.
- IGRA:$CD4^+$ cell ratio may be a useful marker with good diagnostic performance.

Performance for the diagnosis of active TB in HIV-negative adults in high-burden countries

- IGRAs have poor specificity in HBCs.
- Quantitative IGRAs may distinguish active TB from LTBI.
- Current commercial IGRAs are not designed for HBC use and could be tuned.

Performance for diagnosis among symptomatic children suspected to have active TB

- IGRAs offer sensitivity and specificity advantages over the tuberculin skin test (TST) among symptomatic children, especially among malnourished or HIV-infected children.

Performance among children who are asymptomatic contacts of active TB

- IGRAs offer a sensitivity advantage over TST.
- IGRAs are not influenced by *Bacillus* Calmette–Guerin in HBCs.

Performance for prediction of onset of active TB in high-burden countries

- IGRAs do not clearly correlate with risk of progression from LTBI to active TB in HBCs.

Performance for prediction of outcome of therapy for active TB in high-burden countries

- IGRAs do not have a reliable prognostic capability for TB patients undergoing treatment, demonstrating variable responses under treatment.
- The understanding of IGRA conversions and reversions is incomplete.

Conclusion

- The clinical utility of currently available IGRAs for the diagnosis of TB in HBCs is minimal. However, the assays may be optimized for this application in the future.
- Symptomatic children may benefit from the advantages of IGRAs over the TST.
- Truly important new diagnostics will require minimal laboratory infrastructure.
- Future research into the IGRA for HBCs will include simplification, optimization and application in special populations.
- IGRAs are not able to replace currently available TB diagnostic tools in HBCs.

The focus of further IGRA research in HBCs should address specific clinically relevant diagnostic questions in which IGRA might offer significant advantages over conventional methods. These populations should include HIV-infected, or otherwise immunocompromised, persons, children, smear-negative and extrapulmonary TB, healthcare workers and household contacts. Further work in cost reduction and simplification must be prioritized if the benefits of IGRAs are to be realized by the populations that are in most need of a new diagnostic test. One approach could be centralization of the technique through transportation of partly processed plasma samples to referral laboratory facilities.

The IGRA may be better suited for application in HBCs after it has been optimized and tuned for this setting. We can not assume without further investigation that a test designed and marketed for low-burden counties will have a significant clinical benefit in HBCs. Equally, we can not discard a potentially useful technology that may evolve into relevance for HBCs through research directed towards appropriate diagnostic questions.

In terms of having a significant impact on TB control in HBCs, it is unlikely that current IGRAs will contribute to this. Further research will be needed to produce a test with good performance and minimal infrastructure requirements, which may have an enormous impact on TB control, saving 392,000 lives per year [35].

Future perspective

The IGRA platform will be widely adopted as an alternative to the TST in low-TB-burden countries. In its current format, it will not offer significant benefit in HBCs and will not be used. However, further research may lead to optimization of these assays by inclusion of different antigens, use of more appropriate cutoff values for positivity, or improvement in reproducibility, making them somewhat more appropriate. However, the test with the largest impact will have significantly lower infrastructure requirements than the IGRAs. Influential future TB diagnostics will incorporate point-of-care immunochromatographic antigen testing from urine or saliva and will be specific for active TB, with incorporated drug-resistance detection.

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