Interferon gamma release assays for tuberculosis and their potential as efficacy markers for intervention trials
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9. DISCUSSION
9.1. Overview

The body of work in this thesis presents original work using different epidemiologic methodologies. The objective were to: evaluate the performance of IGRAs in the diagnosis of LTBI and active TB disease in adults and children; report on the potential usefulness of IGRAs as biomarkers of disease progression; and rigorously assess the utility of an IGRA as a predictor of treatment efficacy in LTBI and active TB disease in a high TB burden setting. It contributes relevant data to the slowly increasing pool of important IGRA data from settings of high TB burden. This is important, as highlighted earlier, because only a few IGRA studies have come from this type of high burden LMIC setting, as opposed to the abundance from low TB burden HIC settings. Findings here contribute to the notion of differential IGRA test characteristics by setting. The specific findings here are discussed broadly particularly in relation to test performance and changes in results in response to therapy for LTBI and active disease with serial testing.

9.2. Identification of population at risk for LTBI by IGRAs compared to TST

There is an enormous reservoir of persons with LTBI at risk of progression to active disease, and treatment for LTBI prevents progression to active TB disease. Therefore, identification and treatment of LTBI in the groups with the highest risk for progression to active disease is important for TB control efforts. The proportions of TB cases due to transmission within households and the community vary by reports and settings. However, a significant proportion of new TB cases identified in low and high burden settings are the result of recent TB transmission.[1-4] In The Gambia, over 25% of newly diagnosed TB cases give a history of contact with a TB case. [5] New and unrecognised cases drive TB. With transmission usually occurring before diagnosis of the index case, household contacts of TB patients in high burden settings are at very high risk of developing TB in the Gambia and elsewhere. [4, 6] Diagnostic delays contribute to this risk in high TB burden settings. For example, the mean duration of symptoms in cases before TB diagnosis is 9 weeks in The Gambia. [7] This risk of active disease following transmission in household contacts is even higher in those with immunological evidence of LTBI.[8] However, as highlighted earlier, 30-50% of exposed contacts without immunological evidence of LTBI also progressed to active TB, which implies suboptimal sensitivity of the existing diagnostic test (TST) for LTBI. With the foregoing, a test capable of identifying individuals at highest risk for disease progression would greatly aid in the selection of individuals who would most benefit from preventative therapy. The need to find a replacement for TST because of its suboptimal sensitivity, specificity and predictive value provided the drive for development of IGRAs as the first new diagnostic tool for LTBI in over a century.
The absence of a gold standard for the diagnosis of LTBI has made evaluation of IGRAs difficult. Therefore, assessments of their performance in the diagnosis of LTBI has involved proxies including proven TB exposure (classified as exposed or not) or different gradients of TB exposure according to sleeping proximity to the index TB case. The studies outlined in chapters 2-3, describe the performance of a variety of IGRAs compared to TST against a reproducible surrogate for TB exposure-the “Gambian TB exposure gradient” [9, 10]. This reproducible TB exposure gradient as a surrogate is a useful tool for assessing the performance of new tests for LTBI. As shown in chapter 2, our pre-commercial version of the ELISPOT-based IGRA, a commercial whole blood based IGRA and TST are all be able to detect *M. tuberculosis* infection. Since exposure to *M. tuberculosis* was associated with increased likelihood of infection, we conclude both IGRAs and TST perform similarly in response to a TB exposure gradient. IGRAs are considered to have an advantage over TST in adults because their specificity is not compromised by BCG vaccination. [11] However, this does not seem to be the case for TST in high burden TB settings where BCG vaccination soon after birth is the norm. In The Gambia and similar settings, TST like IGRA results are unaffected by BCG status. [10, 12-14] In low burden settings, the converse is true.[15, 16] In addition, IGRAs correlate less with TB exposure compared to TST across all age groups in The Gambia [10, 12, 17, 18], which is different from findings in low TB incidence settings.[19-21] The consensus from the pooled data from high burden settings including data from work in this thesis suggests sensitivity of IGRA and correlations with exposure are not consistent between low-incidence and high-incidence TB settings.[22, 23]

Children, especially those <5 years and particularly those <2 years, are a subgroup of TB contacts with a much higher risk of progression to active TB disease compared to adults. [24] The likelihood of a childhood TB contact progressing to active TB following contact with a case is closely related to child’s age since within 5 years of acquiring LTBI, ~40% of exposed children aged <12 months progress to active TB without any intervention, compared with 10% of children >10 years and 5% of adults. [24, 25] A significant proportion of children who progress to active disease usually do so within 2-12 months of acquiring the infection.[26] Although childhood TB is estimated to be 15-20% of the case load in many TB endemic settings, it is poorly notified in most high burden TB countries where reports of smear positive TB in those <14 years is typically <5%. This is not surprising because children at this age mostly have smear negative and extra pulmonary manifestations of TB. [27] The paucibacillary nature of TB disease in children is what makes diagnosis of active TB extremely difficult so that bacteriological confirmation is the exception rather than the rule. Therefore, the diagnosis of active disease in children <14 years typically relies on a combination of circumstantial evidence-contact history, clinical symptoms and radiological findings, as well as consideration of LTBI assessed by TST. [28] This highlights the need for adjunctive tests that can help with diagnosis in the absence of bacteriological confirmation. In very young children, TB tends to disseminate more frequently because their maturing innate and adaptive immune responses are unable to sufficiently contain the infecting
pathogen.[29] In recognition of this heightened risk and resultant morbidity and mortality associated with TB in children, current WHO guidelines recommend prophylactic treatment for TB-exposed children age <5 years if TST is unavailable. However, National TB Programmes have mainly focused on identification of infectious adult smear positive TB cases. The absence of an accurate test for the identification of those who are *M. tuberculosis*-infected to focus programmatic delivery of preventive therapy has not helped the TB prevention agenda, especially for children. IGRAs came with a promise of utility in this role with the potential also of being a useful adjunct in the difficult process of diagnosing childhood TB. Unfortunately, there is still very limited data on the performance of IGRAs in children especially in high TB incidence settings where there is also a significant burden of childhood TB. Conclusions from data obtained from children in low incidence settings is similar to that reported for adults i.e. IGRAs have higher specificity than the TST, better correlation with surrogate measures of exposure to *M. tuberculosis* and less cross-reactivity than TST on account of previous BCG vaccination.[16, 30]

These conclusions are at a variance with findings in chapter 3 where the 2 widely available commercial versions of IGRAs were compared with TST. We show in this chapter, that the TST is not confounded by BCG vaccination status despite the practice of universal BCG vaccination at birth and this has been a consistent finding in Gambian children.[12] Although all 3 tests had the ability to detect *M. tuberculosis* infection, TST was superior to QFT-GIT and T-SPOT in its correlation with a surrogate measure of TB exposure just as reported earlier in adults and children with our pre-commercial ELISPOT assay.[10, 12] Combination of an IGRA with TST improved the correlation of both IGRAs with TB exposure especially for the T-SPOT. Modelling suggests this observed improvement was the result of a gain of ~10% sensitivity, which is also associated with a slightly greater specificity loss. The benefit of this finding is currently unclear. The utility if any, of IGRA and TST testing in combination for identifying those children with the highest risk of progression to TB in high burden settings requires more research. Unfortunately, there is currently insufficient data on the predictive value of a positive IGRA result in adult and children with LTBI. Data from longitudinal studies here, in adults, show the largest number of latently infected TB contacts that became TB cases were those with LTBI defined as a positive test by either IGRA or TST. Taken alone, each test was only able to correctly identify just under half of all those who eventually developed active disease compared to over 70% for both. [8] More longitudinal studies are required to determine if this is the case with children. However, this provides arguments in support of encouraging TB control programmes to implement preventive treatment as recommended by WHO.[27]

In a systematic review of the performance of IGRAs for LTBI in children, the authors found very limited data especially from high TB incidence countries—there were only 3 papers including data in this thesis (chapter 3).[31] That review forms the basis of the WHO recommendation against the replacement of TST by IGRAs for diagnosis of LTBI. It is possible specific subgroups such as the very young and HIV infected children may have benefit from
a test without the disadvantage of anergy seen with TST in this group but available data are inadequate to provide an answer.

People living with HIV co-infection and LTBI have the highest risk of progression to TB without intervention.[32] Though the body of work described in this thesis did not have objectives related to HIV status, there were still a number of HIV co-infected participants among cases and contacts despite the low burden of HIV in this setting. Current recommendations require Isoniazid Preventive Treatment (IPT) for all HIV co-infected persons with LTBI on exclusion of active TB disease and TST is not a requirement for initiating IPT. [33] HIV infected persons represent another group that will benefit from a test that can identify those at risk of progression from LTBI to TB and that can distinguish between LTBI and active disease. There is evidence of significant benefit from chemoprophylaxis in TST positive HIV co-infected persons compared to those with a negative TST. [34, 35] Unfortunately, the optimal test for identifying HIV-infected persons who would benefit the most from IPT is an unanswered question despite the recommendation above. The immediate benefit of a test with these characteristics is reduction of over treatment for LTBI in a population with a higher risk of drug interactions and hepatic toxicity. Unfortunately, IGRAs are unable to provide these advantages, as they had similar sensitivity to the TST. In HIV infected compared to non-HIV infected persons, the ELISPOT testing platform appears less affected by the HIV-associated anergy compared to the ELISA platform and TST. [36, 37] Current guidelines recommend IGRAs should not replace TST for diagnosis of LTBI in adults and children living with HIV infection in LMICs.[35]

9.3. IGRA with or without TST in active TB disease

Although IGRAs were developed primarily to replace TST, they are now considered as adjunct tests to help with the diagnosis of active TB disease. Furthermore, in the absence of a gold standard for LTBI, studies have often extrapolated sensitivity in active TB to performance in LTBI.[35]

The sensitivities of IGRAs are significantly superior to TST for proven active TB disease as proxies for LTBI but they are just as poor as TST in their specificity.[22] High TB incidence settings have relatively high prevalence for LTBI. This coupled with the inability of these tests to discriminate between LTBI and active disease is a significant contributor to this poor test characteristic, as reported in chapters 3 and 4. Reports, predominantly from high incidence settings suggest the ELISPOT based test platform is superior in performance to the whole blood ELISA-based one. [38, 39] We also had previously reported lower sensitivity for QFT-GIT compared to an in-house ELISPOT in The Gambia but this was an earlier generation kit without a positive control tube. For the T-SPOT and 3rd generation QFT-GIT as reported in chapter 4, we found high and similar sensitivities for both IGRAs contrary to findings from other similar settings. In analysis of pooled data in the WHO policy statement for LMICs with
high TB burden, the authors report a non significant trend towards a higher sensitivity for the ELISPOT compared to the ELISA platform in active disease.[35] IGRAs do not correlate with smear grade or severity of radiological changes in active disease. The influence of Mycobacterial strain differences on sensitivity of commercial IGRAs is not well known. Phenotypic differences between cases and contacts infected with different *M. tuberculosis* strains provide some pointers in this direction.[40, 41] In this thesis, evidence for a likely variation in IGRA sensitivity and differential response to ESAT-6 and CFP-10 according to infecting Mycobacterial strain described in chapter 4 needs further investigation.

The diagnosis of TB is difficult in groups such as HIV co infected persons and in children especially those <5 years. IGRAs in some settings are adjuncts to diagnosis in these special groups. The results in children even in low incidence countries have been conflicting. QFT-GIT and T-SPOT identified a similar number of active cases in children compared to TST in a study by Kampmann et al [42] in the United Kingdom. In addition, the QFT-GIT had better sensitivity than the T-SPOT. Other studies on children in similar settings report the opposite-IGRAs identified more TB cases and were more specific than the TST. [43, 44]

The consensus including limited number of reports from low income countries is that IGRAs have no role in the diagnostic work-up for active disease both for HIV infected and non infected persons.[35] Emerging reports suggest IGRAs may have a use in the diagnosis of TB if disease site-specific lymphocytes are used, as may be the case in TB meningitis, pleural effusion, etc where it is hypothesised *M. tuberculosis*-specific T cells are present at a much higher frequency at the site of disease compared with the periphery.[45, 46] However, the health system cost specifically for laboratory infrastructure, supplies and training of personnel may limit the use of IGRAs on site specific specimens needed to diagnose extra pulmonary and other forms of TB in resource limited settings.

The search for new biomarkers that can distinguish between active TB and LTBI especially in children and immunosuppressed persons remains a research priority.

**9.4. Immunologic basis for IGRAs**

The CD4+ T-cell plays a central role in mediating interactions between T lymphocyte subsets and *M. tuberculosis* infected macrophages which is essential to the adaptive immune response to TB.[47] In this role, CD4+ T-cells dominate but are supported significantly by other T lymphocyte subsets; mainly CD8+, CD1-restricted T and γδ T-cells.[48] Animal and human studies have confirmed this.[49, 50] The HIV associated depletion of CD4+ T-cell number and function that is associated with progressive primary infection and reactivation TB provides further evidence in support of their role in protection against TB.[51] These T-cells produce effector and regulatory cytokines mainly IFN-γ; and others including TNF-α and IL-12 which activate macrophages to kill intracellular Mycobacteria. In particular, IFN-γ is a
secreted effector cytokine by CD4+ cells.[48, 52] IGRAs measure the production of IFN-γ following stimulation with *M. tuberculosis* specific antigens in recognition of the key role played by this cytokine in the adaptive immune response as well as in TB control.[53, 54] As reported in chapter 5, the predominant cell type involved in producing the IFN-γ measured by IGRAs and how this may differ by the *M. tuberculosis* antigen is important. Our finding that CD4+-depletion significantly reduces the response to ESAT-6 demonstrates CD4+ T-cells are the key cellular source of IFN-γ in TB cases in this setting. Although ESAT-6 and CFP-10, the 2 most commonly used antigens in IGRAs, are both secreted by the ESX-1 secretion system (also known as Region of Difference-1 [RD-1]), and have 40% homology, a combination of results from both is utilized in IGRAs as this provides better sensitivity than seen with either antigen alone. [55, 56] As data in chapters 4, 5, 7 and 8 shows, qualitative and quantitative ESAT-6 responses are higher than for CFP-10 in Gambian TB cases and household contacts. Evidence for this as shown in chapter 5, is that CD4+ T-cell depletion significantly affected IFN-γ production compared to CD8+ T-cell depletion. This finding confirms CD4+ ESAT-6 specific effector cells are the main source of IFN-γ measured by IGRAs. [57, 58] The converse is the case in reports from other settings and variations in the responses to ESAT-6 and CFP-10 by HLA-DR types in individuals are well described. [59-61] On the other hand, we found CD4+ and CD8+ lymphocytes both produce the IFN-γ in response to CFP-10 compared to that for ESAT-6 that predominantly comes from CD4+ cells. Since all of these responses are genetically determined or regulated, as described for ESAT-6 in West Africa [62, 63], these findings may have significant impact on the performance and use of IGRAs and even contribute to the differences currently observed in LMIC high incidence TB compared to HICs low TB incidence settings.

### 9.5. Reversion of IGRA with antituberculosis treatment in active disease

Although, the anti-TB drug development pipeline is at its largest since the discovery of Rifampicin in the 1960s, the absence of easy to use biomarkers for treatment efficacy has hampered the process significantly. To prove efficacy now, at least 2 years of follow-up is required after completion of treatment to identify relapse cases. Other biomarkers in use include crude correlates of drug efficacy such as early bactericidal activity and 2-month sputum culture conversion.[48] Taken together, these limitations contribute to the exorbitant costs and extended period required for efficacy trials. IFN-γ production held promise as a proxy for monitoring treatment and predicting relapse because of its role in the immune response to TB. Reports of IFN-γ and other cytokine profiles like IL4/IL4δ2 ratios varying with treatment of LTBI and active disease reinforced this promise.[64-68] In chapter 7, the kinetics of change of IGRAs as an immunological biomarker for treatment efficacy in active TB disease is examined.

The data in this chapter confirms the occurrence of qualitative and quantitative reversion of a positive IGRA in newly diagnosed cases of active TB. [57] However, the reversion seen in
TB cases did not occur reliably and consistently in the majority of cured individuals, implying IGRAs in their current form have limited clinical utility as a surrogate marker of treatment efficacy in active disease. One major limitation of this and other studies into IGRA reversion in active disease is the uncontrolled design that was essential since it is unethical to allocate TB cases to any placebo treatment. Other authors have also described this inconsistent reversion in LTBI and disease. [59, 67, 69, 70] The conclusion of a review of available data was that the reversion from positive to negative IGRA values occurs in a minority of treated patients, and the utility of IGRA reversion has only speculative value as biomarker for treatment efficacy. [71]

9.6. Reversion of IGRAs with treatment efficacy in LTBI?

As highlighted above, reports from uncontrolled studies in LTBI and active TB disease suggest treatment induced changes in IFN-\(\gamma\) may be a proxy marker for treatment efficacy in LTBI. As described in chapter 5 and section 9.4 above, antigen-specific Th1-type IFN-\(\gamma\)-secreting CD4+ T cells and CD8+ T cells are the main sources for IFN-\(\gamma\). In LTBI, IFN-\(\gamma\) produced in response to stimulation with ESAT is from CD4+ T-cells while for CFP-10, both CD4+ and CD8+ T-cells contribute.[58] A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of pathogenic processes or responses to therapy.[72] It has correlates with the activity of the disease process and/or provides feedback on treatment efficacy. A widely held theory is that the frequency of effector T-cells producing IFN-\(\gamma\) in TB is determined by antigen load in vivo. [56] As reported in chapter 6, rising IFN-\(\gamma\) measured by IGRAs in LTBI appeared associated with progression to active disease supporting the notion of Mycobacterial antigen loads driving effector T-cell frequencies.[73] Reports from animal and human models show some correlation between quantitative IGRA results and disease progression and pathology. [74, 75] Chapter 8 in this thesis is a randomised blinded controlled trial of Isoniazid versus placebo for the reversion of a positive IFN-\(\gamma\) ELISPOT response in adult TB case contacts with LTBI. A randomised controlled trial is necessary in the context of reports describing qualitative and quantitative reversions following chemoprophylaxis for LTBI in uncontrolled studies [59, 76] and spontaneous conversions and reversions of IGRAs in cohorts of untreated latent TB infection [77, 78]. It is the first trial to our knowledge, which formally assesses the role of IGRAs as biomarkers for response to treatment for LTBI. It also takes into account genetically determined differences in drug metabolism among participants.

That a course of INH taken regularly can reduce significantly, the rate of later development of active TB disease is a proven fact.[34, 79] The bactericidal effect of INH against \(M.\) \(tuberculosis\) underpins the efficacy of IPT taken for 6-9 months. The pathogen numbers found in LTBI are relatively small so only one drug can be used to treat LTBI for 6-9 months while a combination of 2 drugs can be taken for a much shorter length of time. An efficacy
biomarker for LTBI should therefore change signal with successful INH treatment of true *M.
tuberculosis* infection. Data from chapter 8, show significant decline in quantitative and
qualitative IGRA readout in the INH and placebo arms of the trial over time. Surprisingly, the
observed reversion was unrelated to whether the participants were on INH treatment. This
implies that the natural decline of T-cell frequencies and not therapeutic interventions
against LTBI are responsible for the changes observed in IGRA. INH in treatment of LTBI and
indeed active disease exerts a bactericidal action that eliminates or significantly reduces
Mycobacterial load. With the foregoing, T-cell frequencies following stimulation with *M.
tuberculosis* antigens in IGRA measured as IFN-γ produced do not correlate as well with
bacterial burden as previously thought. In addition, the IFN-γ cytokine profile is not
discriminatory. The current thinking is different cytokine profiles of CD4+ T-cells are
associated with Mycobacterial load during the continuum of host-pathogen interaction that
characterizes human TB. [80] The IFN-γ and CD4+ T cell responses while crucial for
protection against TB are not sufficient representation of the scale and breadth of the
adaptive immune responses to TB. [52] Indeed, apart from well described association
between CD4+ T cell depletion and elevated susceptibility to tuberculosis in HIV-infected
individuals[81], increased susceptibility has been seen with deficiencies in IL-12 and IFN- γ
signalling pathways[82]; and treatment with anti-TNF-α medication[83] suggesting a role for
other cytokines. In addition, other CD4+ T-cell subtypes with capacity to produce single or
multiple cytokines seem associated with protective TB immune responses. [84] This
suggests that combined cytokines in blood or gene expression profiles rather than single
cytokine response may be better candidate biomarkers for protection, disease and drug
monitoring. [48, 52] Indeed, from work here and elsewhere, combination analyses from
data with the IGRA contained or novel TB antigens are rapidly providing candidate cytokine
profiles for further evaluation. For example, Sutherland et al report TNF-α, IL-12(p40) and
IL-17 following TB10.4 stimulation resulted in 85% correct classification into active TB or
LTBI in contacts, and 74% correct classification into TST positive or negative was achieved
with IFN-γ alone (69% ESAT-6 and CFP-10) following TB10.4 stimulation. Moreover there
were higher frequencies of IFN-γ+, IL-2+,TNF-α+ CD4+ T cells in subjects with active TB
disease compared with their household contacts.[85] Others have reported that IL-15 and
MCP-1 accurately identified 83% of active and 88% of latent infections and their
combination was accurate in distinguishing persons with active TB from persons with LTBI.
[86] Considering these multiple profiles, there is the possibility that the T cell response to
LTBI is simply too complex to provide a reliable efficacy biomarker even when we gain
better understanding of the human adaptive response to TB.

Regardless of this, chapter 8 demonstrates that the clinical trial methodology utilised here
can serve as a model for future biomarker evaluations in high TB incidence settings.
References