Interferon gamma release assays for tuberculosis and their potential as efficacy markers for intervention trials

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10. SUMMARY

Chapter 1

This introductory chapter provides a review of tuberculosis epidemiology with emphasis on the burden of active TB disease and latent TB infection (LTBI). It also summarises the adaptive immune response to TB and outlines the gaps that exist in our knowledge of the host-pathogen interaction. It provides an overview of the current challenges in TB control efforts with highlights particularly on the existing limitations in the identification/diagnosis of LTBI. It examines TST as the most widely available diagnostic test for LTBI and its limitations. It addresses the newly developed TB IGRAs, highlights the paucity of data regarding test performance and characteristics in high TB burden setting and draws attention to the areas requiring additional research to improve or validate them as adjuncts in the diagnosis of active TB. Furthermore, their performance in high risk groups-children in this case and the possibility of a role in monitoring and assessment of treatment efficacy in active disease and LTBI were examined

Chapter 2

Although all IGRAs essentially measure the production of IFN-γ production by sensitized T-cells in response to stimulation by relatively specific *M. tuberculosis* antigens, their performance may vary with not just the populations tested but also the technique of IFN-γ detection. Chapter 2 describes the test performance characteristics of an IGRA that uses an enzyme linked immunospot (ELISPOT) assay technique compared to a commercial version that utilises whole blood samples-QuantiFERON-TB Gold in tube (QFT-GIT, Cellestis Limited, Carnegie, Australia). Using culture confirmed active TB as gold standard, sensitivity of our in-house ex-vivo ELISPOT based IGRA was significantly better than for the ELISA based commercial IGRA. For LTBI in household contacts, both tests correlated well with a validated gradient of recent exposure to *M. tuberculosis*. The specificity of either test was unaffected by BCG vaccination. However, significant discordance was observed between both IGRAs as well as between IGRAs and TST and the reasons for this remains largely unknown although differences in test formats, BCG vaccination and better specificity for IGRAs compared to TST have been hypothesized.

Chapter 3

There is very limited data on the performance of IGRAs in children despite recommendations for the use of IGRAs in any setting that TST is required in low burden settings. This chapter provides results from a three-way comparison of the performance of 2 IGRAs and TST in a high risk population-childhood TB contacts. It also describes the performance of IGRAs or TST in combination for diagnosis of LTBI. The TST was superior to both IGRAs in its correlation to a gradient of *M.tuberculosis* exposure. All tests were unaffected by prior BCG vaccination. The performance of both IGRAs in relation to the gradient of TB exposure was improved especially for T-SPOT when combined with TST.

Modelling suggests that an approximate 10% sensitivity benefit for using the TST and an IGRA in combination is associated with a slightly greater specificity loss.

Chapter 4

It is now acknowledged that test characteristics of IGRAs for LTBI and active TB disease vary by setting i.e. high TB versus low TB incidence countries. The reasons for this are not fully understood. As part of evaluating the performance of the widely available commercial IGRAs for LTBI using proven TB disease as a surrogate standard, this chapter compares the sensitivities of the T-SPOT and QFT-GIT against a gold standard of culture positive TB. It also examines the possible role of the infecting strain of Mycobacteria (*M. Tuberculosis* vs. *M. africanum*) on test results. Both IGRAs showed high sensitivity in active TB disease compared to TST. There were sensitivity gains when each IGRA was combined with TST but it is unclear how this testing strategy can be used in high TB incidence settings. There was a trend towards lower sensitivity, lower median spots with T-SPOT and IFN-γ concentrations with QFT-GIT in cases with *M. africanum* compared to *M. Tuberculosis* but this did not reach statistical significance. The role of the infecting Mycobacteria strain on IGRA test characteristics requires further investigation.

Chapter 5

The adaptive immune response to infecting *M. tuberculosis* and the IGRAs revolve around the key role played by IFN-γ secreted by sensitized T cells. Identification of the major T lymphocyte subset responding the *M. tuberculosis* antigens during infection or disease may influence the interpretation of interferon-gamma release assays. This chapter reports on the assessment of the contribution of CD8+ and CD4+ lymphocytes to the ESAT-6 and CFP-10 antigen-specific IFN-γ response using the ELISPOT assay in active TB disease and LTBI. The ESAT-6 responses were higher than for CFP-10, and CD4+-depletion significantly reduced the response to ESAT-6 in TB cases compared to contacts. The response of the CD8+-depleted cells to ESAT-6 in TB cases was also higher than that of the contacts. Taken together, the frequency of ESAT-6 specific IFN-γ producing effector cells is higher in TB cases and their contacts, and these come mainly from CD4+ T lymphocytes. Thus, conditions with significantly reduced CD4+ T cells will impair the sensitivity of IGRAs.

Chapter 6

The absence of biomarkers or tests that can identify the subgroup of persons with LTBI who will benefit the most from preventive treatment currently impairs assessment of therapeutic interventions against TB contacts particularly in high TB burden settings. This chapter is a case report on qualitative and quantitative changes in the ESAT6/CFP10 (EC) IFN-γ ELISPOT with LTBI following exposure to a TB case. The observations here suggests the need for further investigation to confirm if rising EC IFN-γ ELISPOT counts can predict progression from LTBI to active disease.

Chapter 7

We have already observed that there can be progressive increase in quantitative EC ELISPOT counts leading up to active TB disease in a household contact of a TB patient. This chapter examines the kinetics of the ELISPOT counts following anti-TB treatment. Significant decline in the proportions of IGRA positive cases was seen and this occurred mostly at the 1- and 6-month of treatment. This qualitative decline was mirrored by significant quantitative changes in both antigens tested for in the IGRA. Significant decline (reversion) was associated with lower pre-TB treatment ELISPOT counts, as those without demonstrable reversion mostly had higher counts at the start of treatment. Although, TB treatment induces significant qualitative and quantitative reversion of a positive in-house IGRA in newly diagnosed active TB disease, the EC ELISPOT has limited clinical utility as surrogate marker of treatment efficacy because the reversion does not occur reliably in the majority of cured individuals.

Chapter 8

As described in chapter 6, IGRAs may have some correlation with *M. tuberculosis* antigen load, which suggests limited utility of qualitative and quantitative reversions of IGRA as surrogates for efficacy of therapeutic interventions in LTBI and active TB disease. Indeed, the EC ELISPOT reversion following treatment in TB cases occurred in those that had relatively lower pre-treatment ELISPOT count. Since such low counts are likened to what we have measured in LTBI, this chapter evaluated if IFN-g ELISPOT response to EC could be used as a biomarker of INH preventive treatment for LTBI. The study is the first randomized, blinded and placebo controlled clinical trial of INH preventive treatment for reversion of a positive EC ELISPOT (IGRA). Significant qualitative and quantitative reversion of the EC ELISPOT was seen over time but was not related to treatment allocation i.e. there was no difference in reversion seen in intervention compared to control arm. Also, there was no interaction between study arm i.e. INH treatment, acetylator status and EC ELISPOT results.

Chapter 9

This section of the thesis is a general discussion of the data presented in the earlier chapters describing the performance of IGRAs including comparisons of the different techniques of IFN-γ detection as well as comparison with TST for diagnosis of LTBI and active TB disease. It also considers the evidence generated in relation to the hypothesis that IGRAs could play roles as candidate biomarkers for susceptibility or predictors of treatment success in active disease or efficacy during preventive treatment for LTBI. All of these are examined in the context of a low resource high TB burden country setting.