Reviewer's report

Title: Improved Sensitivity of an Interferon-Gamma Release Assay (T-SPOT.TB) in Combination with Tuberculin Skin Test for the Diagnosis of Latent Tuberculosis in the Presence of HIV Infection

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Reviewer: Graham H Bothamley

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This paper describes the use of an immunospot test in frozen cells collected from patients with HIV who developed tuberculosis subsequently.

Activated immune cells tend to be larger and have higher water content. Freezing and thawing of such cells is more likely to lead to lysis than for resting cells. The process of storing cells may therefore have specifically affected the population that is normally examined in the short incubation periods of the chosen test.

Major comments

The purpose of this study in clinical terms needs to be more clearly defined. If the purpose is to define who should receive preventive treatment, then the samples need to be tested fresh at the point of HIV diagnosis in a standard test. This cohort would also be helpful to define whether repeated testing is of value. If the nature of reactivation is important, then the reason for negative tests in those who developed tuberculosis needs further assessment, e.g. by the measurement of Treg cells in a cell sorter. Were samples initially positive and then became negative as the time point of active tuberculosis approached? A comparison with the demographics of those who did not develop tuberculosis might be helpful to elucidate whether a combined negative elispot and tuberculin skin test were of pathogenetic significance.

The first step in such a study is therefore to validate the method, to compare fresh with frozen lymphocytes and time of incubation necessary to obtain comparable results. There are data in the literature looking at CD8+ interferon-gamma responses to peptides from HIV in elispots from fresh and frozen cells. The time from collection of the sample to freezing appears to be especially important and should be recorded in the manuscript under “according to a specific protocol” (p7).

Whilst reactivation of latent infection seems most likely, absence of exposure to tuberculosis and a lack of clonality in HIV-related Mycobacterium tuberculosis strains would be important to document. Even though the country has a low incidence of tuberculosis, some community groups have a much higher incidence of tuberculosis. The most important correlation between a positive elispot test is with duration of exposure to tuberculosis, such as time spent in an area with a high incidence of tuberculosis - which needs to be included in the variables listed
For an international audience, the “grey” zone of values recommended by the US FDA should be included. The actual values of the number of spots could be usefully documented. The term “fair mitogen control” needs to be defined in the methods – was this a positive control <20 spots but greater than a defined value, or between 20 and 50 spots?

As the number of spots varies among individuals, correlation with CD4 count by Spearman’s test is not sensible – the figure speaks for itself and is sufficient.

Other comments
Is an elispot performed at entry into the cohort? This would be potentially more valuable in indicating whether preventive treatment in those with a positive test might be beneficial. Or is chemoprevention recommended on the basis of the tuberculin skin test? Did any member of the cohort receive chemoprevention?

**Level of interest**: An article of importance in its field

**Quality of written English**: Acceptable

**Statistical review**: No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests**: I declare that I have no competing interests