Elispot-T assay to monitor treatment response of latent Tb

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A. Study Purpose and Rationale

Tuberculosis (Tb) remains one of the leading global public health problems, with an estimated one-third of the world's population currently infected. Out of those about 9 million progress each year to active disease [1]. Despite effective control programs in industrialized countries, a vast pool of individuals infected with latent tuberculosis infection (LTBI) remains undiscovered. They continue to be at risk for progressing to active disease, especially if they become immuno-compromised later in life [2]. Unfortunately, diagnosis of latent Tb is severely hampered by the lack of a reliable, standard diagnostic test. Until recently, this would solely depend on the tuberculin skin test, whereby a predefined amount of purified protein derivative is injected according to the intradermal Mantoux method [3]. Major shortcomings of this test include that the purified protein derivative of tuberculin contains many antigens that are shared with other mycobacteria, which the skin test is not able to distinguish. This includes infection with atypical mycobacteria ubiquitous in the environment as well as the *Mycobacterium bovis* bacille Calmette-Guerin (BCG) vaccine strain that is used in BCG vaccination in many parts of the world (but not the US) [3, 4].

The skin test is primarily used to screen recent Tb contacts, as part of exposure investigations, as well as health care workers and recent immigrants for their risk of carrying LTBI. After exposure to an index case, the risk to convert to active disease is highest within the first two years and then significantly declines [2]. If a patient has a positive test, he or she will undergo a chest x-ray to rule out active Tb. In an effort to eradicate Tb by aggressively diminishing the pool of latent Tb, current recommendations by the CDC include that any individual who (a) recently converted from a negative to a positive test, (b) has a positive test of unknown duration but comes from an endemic country, or (c) is HIV positive should receive chemoprophylaxis with isoniazid (INH) or alternative regimens if indicated. It is further recommended that INH should be administered over a period of 9 months. However, this may cause significant hepatotoxicity, especially in people with pre-existing liver conditions or people of older age [5]. Given the high false-positive rate of the test, individuals incorrectly identified as having latent infection are subjected to a lengthy and potentially toxic treatment regimen.

In clinical practice, INH treatment for LTB is recommended to the patient, who may refuse. Patients are usually given a 1-month supply of drug. CDC guidelines include that patients treated for latent Tb with INH should be clinically examined once monthly to ask about side effects and look for clinical signs of hepatitis. Routine laboratory monitoring of liver function tests is only indicated if patients have a known underlying liver disease. INH should be withheld if reaching more than 3 times the upper limit of normal levels of transaminases with symptoms, or five times the upper limit of normal levels without symptoms.

However, studies have shown that after treatment for latent Tb is offered, initiation rates are as low as 62% [6]. More strikingly, completion rates amongst health care workers for the recommended 9-months therapy were as low as 12% at Harlem Hospital in New York [6]. In a study among Latino immigrants, the show-rate at the first monthly visit was noted as 81% and dropped to 56% after 6 months [7]. Even when

patients comply with follow-up visits, it is very difficult for the treating physician to follow treatment compliance, and wide-spread use of an available urine drug test is thought to not be cost effective [8].

In cases of successful completion of treatment, the tuberculin skin test will remain positive, thereby precluding any further screening test to monitor people for new exposure to Tb. This is especially problematic amongst individuals living in high or moderately high endemicity countries, as well as health care workers, who are likely to be constantly and repeatedly exposed.

Recently, two new blood tests (Quantiferon Gold, Elispot T assay) have been developed, based on the detection of interferon gamma released by T cells in response to antigens (early-secretory antigenic target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10)) specific to *M. tuberculosis*. A small blood sample is used that is then incubated overnight with an internal positive control, a negative control or the 2 antigens in separate tubes. The Elispot T assay detects individual memory T cells that produce interferon-γ in response to *M. tuberculosis* antigens, using the enzyme-linked immunospot assay.

Both tests are commercially available, with the Quantiferon Gold test already been approved by the FDA, and the newer Elispot T assay currently under review [9]. Evaluation of both tests has been hampered by the lack of a gold standard for latent Tb, making it particularly difficult to assess the true sensitivity. However, multiple studies indicate higher specificity of the new tests with equivalent sensitivity when looking at patients with active Tb [10]. Furthermore, preliminary studies provide evidence that the frequency of *M. tuberculosis*-specific effector T cells declines following successful treatment, therefore potentially allowing for active monitoring of treatment [11, 12].

This study aims to identify the effect of INH treatment on Elispot T assay interferon gamma release in patients who are tuberculin skin test and Elispot T assay test positive.

B. Study design and Statistical Analysis

This is a prospective, observational study to evaluate the utility of a novel blood test in monitoring response to latent Tb treatment, administered as Directly Observed Preventive Treatment (DOPT). The primary endpoint of the study is Elispot test result (antigen ESAT-6 and CFP-10 combined) after 9 months of therapy. The null-hypothesis is that there will be no change in the Elispot assay after treatment with INH in individuals with latent Tb. Subgroup analysis will test change in Elispot assay at 3, 6 and 12 months after initiation of therapy, as well as compare results for the two individual antigens.

To estimate sample size we postulate that the expected magnitude of treatment on the test would be 80%, and that a change in test result in 50% of individuals treated would be clinically meaningful to be significant. In order for the study to have 80% power to detect a 30% difference in test results after INH treatment, we calculate that 54 individuals who are PPD/Elispot positive will need to be recruited. Despite treatment being administered as DOPT, medication non-compliance is expected to be around 20%, requiring an additional 11 subjects, bringing the total to 65 patients.

Descriptive statistics will be used to describe baseline demographic data. Statistical analysis will use the Fisher's exact test and chi-squared test to compare proportions in the pre- and post-treatment groups. Multivariate analysis will be

performed to assess whether particular patient variables are associated with a change in Elispot status.

C. Study Procedure

The study will take place at The Charles P. Felton National Tb Center, Harlem Hospital, NYC. Here, a high volume of individuals is regularly screened after contact with patients with active Tb. This includes health care workers and people from other risk populations (homeless, iv drug users, HIV positive). The test is carried out by intradermal application of 2-TU PPD, RT23, per standard clinical practice. Results are read 48 to 72 hours later. A positive test is defined as >10mm induration. If a skin test returns positive, patients undergo routine chest XR to exclude active Tb. They are then offered treatment with INH, provided either as a monthly supply, or as part of an ongoing study as DOPT.

At this stage, a study coordinator will ask patients whether they are interested in enrolling in the current study. They are asked to provide a blood sample, which is then subjected to the Elispot T assay. If this test also returns positive, they are eligible for the study.

The study starts with a questionnaire, and further blood testing performed at 3 months, 6 months, 9 months (completion of treatment) and 12 months after initiation of treatment. This entails a 5 ml draw of heparinized blood. Blood samples will be analyzed in a dedicated research laboratory. The *ex vivo* Elispot assays will be carried out in duplicate at 37°C. The ESAT-6 and CFP-10 antigens are added at 2.5 μg/ml per sample. Positive test results are defined as forming at least 8 spot-forming units more than the negative (non-stimulated) control wells. The positive control consists of phytohemagglutinin and is set at 150 spot-forming units per well.

Laboratory staff will be blinded to the characteristics of the individuals tested.

D. Study Drugs

No study drugs will be used. However, patients will receive standard treatment with INH for latent tuberculosis as indicated.

E. Medical Device

Not applicable to this study as no medical device will be used.

F. Study Questionnaires

A questionnaire will be administered at the time of enrollment in the study as well as during monthly follow-up visits. This will address relevant clinical and social history at the initial visit, as well as questions related to INH therapy during the follow-up visits.

G. Study Subjects

1. *Inclusion Criteria*
   -Age 18 and older
   -Positive tuberculin skin test and Elispot T assay
   -DOPT

2. *Exclusion Criteria*
   -Active Tb
   -Pregnancy

-Current use of immunosuppressant drugs
-Known immunodeficiency stage
-Underlying liver disease

H. Recruitment of Subjects

Informed consent will be sought by one of the study coordinators.

I. Confidentiality of Study Data

Confidentiality will be protected by standard New-York Presbyterian Columbia Medical Center clinical study procedures, outlined by the IRB and HIPPA guidelines. Blood samples will be processed under a unique study code for each subject.

J. Potential Conflict of Interest

There is no potential conflict of interest.

K. Location of the Study

The study will take place at the Harlem Hospital Center, 506 Lenox Avenue, New York, New York 10037.

L. Potential Risks

The potential risk to the patient is considered minimal. It is related only to repeated blood drawing. Patients may experience discomfort or pain when having blood drawn, infection, minor bruising or bleeding at the needle insertion site, or fainting.

M. Potential Benefits

Although there is no direct benefit to the study participants at this stage, the data derived from this study will ultimately help to better direct the screening and treatment of latent Tb.

N. Alternative Therapies/Procedures

An alternative to not taking part in this study would be to undergo treatment of latent Tb without any follow-up on treatment response. Patients who do not enroll in this study will receive standard care and monitoring according to good clinical practice.

O. Compensation to Subjects

Study subjects will receive free Tb medicine, free Metrocards, free social services, and food vouchers.

P. Costs to Subjects

Patients enrolled in this study will not incur any additional costs as this study is being covered entirely by a research grant.

Q. Minors as Research Subjects

This study does not involve the participation of minors.

R. Radiation or Radioactive Substances

This study does not involve the use of radioactive substances. Patients will be exposed to minimal amounts of radiation as part of standard clinical practice for individuals with a positive tuberculin skin test.

S. References

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