TB:HIV an international prospective observational study

PROTOCOL ADDENDUM 1

Tuberculosis among HIV-positive patients:

an international prospective observational study

Addendum 1.0 dated 14th June 2012

Protocol version 1.0 dated 7th January 2011

Coordinating Office:
Copenhagen HIV Programme (CHIP)
University of Copenhagen, Faculty of Health Sciences
The Panum Institute/Building 21.1
Blegdamsvej 3B
2200 Copenhagen N
Denmark
Tel: +45 35 45 57 57
Fax: +45 35 45 57 58
e-mail: hivtb@cphiv.dk

The purpose of this Addendum is to implement a sub-study on biomarkers for HIV/TB coinfection via collection of plasma samples

1. Background and scientific rationale

The TB:HIV Study is an international, observational, cohort study being conducted in 19 countries in Eastern, Western, and Southern Europe, as well as Latin America. The well-established collaboration includes more than 50 clinics and cohorts with 14 clinics located in the Eastern part of Europe (see main study protocol for details). The study is planning to enroll 1500 patients, where the first 500 are already enrolled.

Tuberculosis (TB) results in a state of immune activation, and even more so, when there is concomitant HIV-infection. Increasing evidence show that severe infections are strongly associated with activation of both inflammation and coagulation, and these two systems seem to be intertwined, meaning that both inflammation can activate coagulation and also vice versa [1]. There is a clear lack of evidence and only few studies done in the field of investigating inflammatory and coagulation biomarkers for HIV/TB co-infection.

Previous research has shown that TB generates a massive immunological response with release of several interleukins (IL), and also induces production of hepatic enzymes involved in haemostatic changes. TB also seems to induce a hypercoagulable state, and has also been shown to be associated with an increased risk of deep vein thrombosis (DVT) [2]. The homeostatic changes which happen during TB disease activate the endothelial intimate layer resulting in increased thrombogenic activity [3]. A smaller study has shown increased levels of fibrinogen, plasminogen activator inhibitor (PAI-1) and platelets together with depressed levels of antithrombin III (ATIII) and protein C levels among patients with active pulmonary TB compared to healthy controls [2].

The role of inflammatory and coagulation biomarkers among HIV-positive individuals with active TB disease is still unclear and further studies are needed. Relevant biomarkers to be measured for this project could include (but are not limited to):

- **Inflammation:** Neopterin, IL-1, IL-6, hsCRP, sCD14, sCD163
- **Coagulation:** Tissue Factor (TF), thrombocytes
- **Anticoagulation/Fibrinolysis:** Thrombocyttes, D-dimer, Plasminogen activator inhibitor 1 (PAI-1)

2. Hypotheses

We hypothesize that biomarkers can be of aid in improving overall TB disease outcome in HIV-positive individuals. We hypothesize that biomarker analyses can address several important research questions, including:

1. Levels of specific inflammatory and coagulation-related biomarkers decline in HIV patients with TB who respond to anti-TB therapy

2. The level of specific biomarkers at time of the TB diagnosis and during treatment for TB may be associated with:
   A. TB disease outcome or risk of relapse
   B. TB IRIS development
   C. anti-TB drug resistance

3. Sub-Study objectives

To collect plasma samples in order to establish a central plasma sample biobank within the TB:HIV study. The aim of such a biobank establishment is to enable that research projects can address important research questions within the HIV/TB biomarker field.

4. Investigational plan

4.1. Sub-Study Design

Plasma samples will be collected from patients included in the TB:HIV prospective study. Samples should be collected consecutively at three different time intervals:

- At baseline (time of TB diagnosis)
- After 1-2 months of anti-TB treatment (completion of intensive phase of anti-TB treatment)
- After 6-9 months of anti-TB treatment (by the end of anti-TB treatment)
- After 12-15 months of anti-TB treatment (after completion of anti-TB treatment).

The collection of consecutive samples will provide the basis for following individual biomarkers and their changes over time during TB disease. This will enable to address several objectives as described in sections above.

4.2. Sub-Study population

The TB:HIV Study sites, who agree to participate in this sub-study will collect plasma samples from consecutive patients (or an unbiased subgroup) enrolled in the study and who agree to provide their plasma. For details on Study population and inclusion/ exclusion criteria please refer to the main Study Protocol, sections 3.2 – 3.4.

4.3. Collection of plasma samples

Plasma samples will be collected and stored for further retrospective analysis. Samples of 2 x 1 ml plasma will be collected and stored locally (frozen at -70°C) at the participating canters according to local rules and regulations. These samples will be drawn simultaneously with samples collected for the routine clinical management of the patient, thus minimizing any patient discomfort and interventions.

The samples will thereafter be shipped to the Coordinating Centre annually (or after a significant amount of samples has been collected), where a central biobank will be established. An affiliated laboratory among participating centres will be then identified for further proceeding of samples and biomarker analyses. The Coordinating Centre has

developed instructions for samples collection, storage and transportation, which will be circulated to all sites participating in the plasma collection.

As TB:HIV researchers physically are located at different European universities and hospitals, datasets containing information from the participants´ medical records and their biologic samples might be analysed at other locations than the TB:HIV Coordinating Centre.

Samples stored will be analysed and destructed in accordance with the legal and/or ethical requirements of the single participating centre/country.

4.3.1. Details on plasma sample collection and storage

- 3 - 5 ml of EDTA blood is collected.
- Blood is separated by centrifugation (e.g. 1.500 g, 15 min.)
- Store 2 x 1 ml aliquots in 1,8 ml screw top cryovials (e.g. Nunc 377267 or similar). Glass vials are not permitted. If not available, please contact the coordinating office.
- The tubes should be clearly labeled with:
    - TB:HIV patient number
    - Date of collection
- Samples should be stored at – 70° Celsius or liquid nitrogen within 4-6 hours of venesection.
  If - 70° Celsius or liquid nitrogen is not available, use - 20° Celsius freezer.
- If plasma have been stored in freezers with temperatures above - 50° Celsius, or if more than 6 hours have passed before plasma has been frozen, this should be clearly indicated when samples are shipped to co-ordination center.
- List of samples available should be sent to coordination center at University of Copenhagen upon request.

4.3.2. Shipment of samples

When ready to ship samples, please contact the Coordinating Centre at University of Copenhagen for precise instructions on handling, packaging and shipping.

Specific regulations exist regarding the international shipment of biological samples derived from HIV-infected subjects. These procedures and regulations for packaging and shipping of infectious samples are outlined in the TB:HIV MOOP. It is the responsibility of the investigator (to be assisted by the courier service and the Coordinating Centre) to ensure that all study samples for international transport are appropriately handled, packed and shipped.

5. Sub-Study Administration

5.1. Sub-Study coordination

The sub-study is coordinated by Copenhagen HIV Programme, University of Copenhagen. Details are outlined in the main TB:HIV study protocol, section 6.1

5.2. Sub-Study governance

The study Coordinating Centre is supervised by the Steering Committee (SC) (please see details in the TB:HIV study main protocol, section 6.2). All research proposals on particular analyses within the HIV/TB biomarker sub-study should be addressed to the Steering Committee. The SC will then discuss all proposals during telephone conferences and/ or face-to-face meetings. Only projects approved by the SC will be fulfilled.

5.3. Funding

The clinical TB:HIV Study is funded through EuroCoord, WP13 for 5 years. Additional funding will be necessary in order to carry out plasma sample collection, shipment and establishment of a central biobank. Staff at the coordinating centre will apply for extra funding through various funds. In addition, assessment of biomarkers for the collected samples requires additional funding. An important component of all new scientific laboratory projects is thus to provide the needed funding to cover laboratory work; this need to come from external sources.

5.4. Publications

The findings from this sub-study, positive, negative or inconclusive, are intended to be published in peer-reviewed journals. The SC decides whether abstracts are to be submitted to conferences, and how the results are distributed if more than one manuscript is to be drafted.

The TB:HIV study group will appear in an appendix in all published manuscripts. Co-authors are selected after a fair evaluation of primarily number of patients entered in to the sub-study and the level of involvement in the drafting of the manuscript. Providing that several manuscripts are to be drafted, a fair rotation among the participating centres of co-authorship slots will be done.

Before publication the SC and co-author group must approve all manuscripts. Co-authorships will be identified using the Vancouver criteria.

5.5. Regulatory and ethical considerations

5.5.1. Regulatory approval

Before sub-study related activities are performed, appropriate local approval of this sub-study and procedure for obtaining informed consent from participants will be obtained according to local and/or national regulations in countries participating in the TB:HIV study, as well as other national regulatory approvals as applicable. The Principal Investigator (PI) at each centre is responsible for obtaining and maintaining this/these approval(s) at all times during the conduct of the study as confirmed by a working agreement.

5.5.2. Patient Informed Consent

The PI or his/her designee will inform the patient of all aspects pertaining to his/her participation in the TB:HIV sub-study on plasma sample collection.

When Patient Informed Consent is required by the local and/or national Ethics Committees, this will be obtained from each patient before any study related procedure is performed.

In accordance with the ICH – GCP Guidelines for the conduct of clinical trials as described in the European Community Directive 2001/20/EC, the following procedure for obtaining Informed Consent will be followed:

- The patient will prior to inclusion in the sub-study be informed verbally by a doctor or study nurse about the sub-study and also receive written information about the sub-study, if required by the national local ethical committee
- The patient will have the opportunity to ask questions
- The patient will be informed that participation is voluntary and that the patient can withdraw his/her consent at any time without any consequence for his/her treatment or future relationship to the clinic/hospital.
- If required by the national or local ethical committee the patient must sign the Patient Informed Consent form before any sub-study related activities can begin

No remuneration will be paid to the participants.

5.5.3. Safety

Study participation involves minimal risk to the participants. Possible risks of having blood drawn include pain, bleeding, bruising, light headedness, fainting, and rarely, infection or a blood clot where the needle enters the body. There are no direct benefits to the participants. However, the benefit of conducting observational research including research on stored samples includes advancing scientific understanding of HIV/TB coinfection and its complications; this knowledge guides international and European treatment recommendations to the benefit of people living with TB and HIV.

To minimise any risks and patient's discomfort, the samples will be drawn simultaneously with samples collected for the routine clinical management of the patient.

5.5.4. Ethical considerations

The TB:HIV sub-study on plasma sample collection and biomarkers analyses is conducted according to the current ethical standards including the WMA Declaration of Helsinki and is submitted to the appropriate regulatory authorities including ethical committees in the participating countries.

Patients included in the TB:HIV sub-study will have a plasma sample taken (3-5 mL) at 3 different time intervals during treatment for TB, patients are not to be exposed to any experimental interventions, nor will the study intervene with the clinical management of the individual patients.

References

1. Levi M. The coagulant response in sepsis and inflammation. Hamostaseologie 2010,30:10-12, 14-16.
2. Turken O, Kunter E, Sezer M, Solmazgul E, Cerrahoglu K, Bozkanat E, et al. Hemostatic changes in active pulmonary tuberculosis. Int J Tuberc Lung Dis 2002,6:927-932.
3. Robson SC, White NW, Aronson I, Woollgar R, Goodman H, Jacobs P. Acute-phase response and the hypercoagulable state in pulmonary tuberculosis. Br J Haematol 1996,93:943-949.