

IMMUNOIDENTIFICATION OF TRYPANOSOMA CRUZI ANTIGENS USING SERA FROM CHRONICALLY INFECTED PATIENTS

By Hílana Patrícia Aristizabal Pineda¹

Medical Doctor, Master student of Master in International Health at Charité – Universitätsmedizin.

January, 2015 Lisbon, Portugal

1. Supervised by Dr. Marcelo Sousa Silva (PhD in biotechnology) Assistant researcher at Instituto de Higiene e Medicina Tropical - Universidade Nova de Lisboa (IHMT-UNL), Portugal. This study was carried out at IHMT-UNL.

Key words: *Trypanosoma cruzi*, Chagas disease, immunoanalysis, immunogenic proteins, Enzyme-Linked Immuno Sorbent Assay (ELISA), Western Blotting (WB)

ABSTRACT

The main objective of this study was to search for the most immunoreactive native proteins of *Trypanosoma cruzi* (*T. cruzi*) by carrying out a serological analysis of anti-*T. cruzi* antibodies in sera from patients with Chagas disease. Following this, an immunoreactive analysis of potential antigens was performed. Next, a group of sera were chosen to search for IgM and IgG antibodies by in-house ELISA, which was optimized in this study. The most reactive sera detected by ELISA were used for a profile analysis of the proteins by western blotting in order to identify the predominant immunogenic proteins from *T. cruzi* that develop humoral immune response in patients in the chronic stage of Chagas disease.

An in-house ELISA optimisation using a total of 57 samples from Latin American chagasic patients living in Europe, was carried out. This optimisation allowed the differentiation between positive and negative samples, and also the identification of the population as chagasic patients by using anti- *T. cruzi* IgM and IgG antibodies. Furthermore, based on the results from the in-house ELISA, anti *T. cruzi* IgG subclasses demonstrated high titres of anti- *T. cruzi* IgG1 and IgG3 antibodies, and the population was classified as patients in the indeterminate or chronic stage of Chagas disease.

The in-house ELISA with crude antigens was compared with two commercially available kits, (ORTHO[®]ELISA and Gold ELISA Chagas REM[®]) which used lysate and recombinant antigens. The former showed more nonspecific reactions than the ELISA with lysate antigens. This clarified the difference between sources of antigens in terms of sensitivity and specificity.

After carrying out a western blotting with the most reactive sera using ELISA for the detection of anti- *T. cruzi* IgG antibody, a pattern of the *T. cruzi* proteins were identified as the predominant immunogenic antigens from the total native proteins of the parasite. These proteins were classified into four groups based on their molecular weight: Group 1: 20-15 kDa band, Group 2: 30-25 kDa band, Group 3: 50-40 kDa band and Group 4: 80-125 kDa band. These proteins can be purified by immunoproteomic analysis and also it is important to test them for cross-reaction with *Leishmania spp* in order to develop a more sensitive and specific diagnostic test, particularly for the chronic stage of the disease. Additionally, they can be developed for use in future vaccines.

Most of the nonspecific reactions between antigen-antibody in western blotting were found to be with proteins with low molecular weights (less than 15 kDa). It is important to carry out future investigations to define if they are actually nonspecific reactions or if they are present in the acute stage of the disease in order to develop a specific test for this phase.