RESEARCH NOTE

Differential Serodiagnosis of Human Infections Caused by *Trypanosoma cruzi* and *Leishmania* spp. Using ELISA with a Recombinant Antigen (rTc24)

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Key words: Chagas disease - cutaneous leishmaniasis - serology - specificity

Chagas' disease and leishmaniasis are endemic protozoan diseases of significant importance in Brazil. Chagas' disease affects 16-20 million of inhabitants in Central and South America, being caused by *Trypanososma cruzi*, a parasite of the family Trypanosomatidae. Parasites of the genus *Leishmania*, that also belong to this family, can cause cutaneous, mucocutaneous, and visceral leishmaniasis. The endemic areas of *T. cruzi* and *Leishmania* infections in Latin America often overlap, including the State of Minas Gerais. A nationwide seroepidemiologic survey of human *T. cruzi* infection carried out in Brazil from 1975 to 1980 showed that Minas Gerais has one of the greatest prevalence (8.83%) in the country (ME Camargo et al. 1984 *Rev Inst Med Trop S Paulo 26*: 192-204).

Serological tests used for the diagnosis of trypanosomiasis, such as indirect immunofluorescence assay (IFA), indirect hemaglutination (IHA), and enzyme-linked immunosorbent assay (ELISA) show significant cross-reactivity with antibodies from individuals infected with *Leishmania* (FG Araújo et al. 1986 *Infect Immun 53*: 179-185, TM Chiller et al. 1990 *Am J Trop Med Hyg 22*: 650-656). Serological cross-reactivity between these two parasites may lead to distorted epidemiological data and pose problems to the diagnosis and further treatment of the patients. The possibility of diagnosing false positive *T. cruzi* infections thus exists, specially in areas where leishmaniasis is also prevalent. For instance, patients in the chronic assymptomatic phase by *T. cruzi* can be misdiagnosed as having cutaneous leishmaniasis when cutaneous lesions caused by non-*Leishmania* agents are present. On the other hand, an individual previously infected by *Leishmania* and presenting cardiac problems other than that caused by Chagas' disease could be falsely diagnosed as infected by *T. cruzi*.

Many attempts have been made to identify specific antigens from *T. cruzi* parasite with a potential use in the diagnosis of Chagas' disease. Whole parasite extracts (WHC Landivar et al. 1992 *J Infect Dis 166*: 1464-1465), soluble antigens (RS Corral et al. 1992 *Am J Trop Med Hyg 46*: 31-38) and glycoproteins (M Schechter et al. 1983 *Lancet 22*: 939-941) are currently being used in serological methods for diagnosis, although specificity in these assays is low. Several recombinant surface antigens (MS Cetron et al. 1992 *Acta Trop 50*: 259-266, A Gruber & B Zingales 1993 *Exp Parasitol 76*: 1-12) as well as cytoplasmic and flagellar antigens (MA Krieger et al. 1992 *Am J Trop Med Hyg 46*: 427-434) of *T. cruzi* have been tested with some success. The polymerase chain reaction (PCR) with specific oligonucleotides (HA Avila et al. 1991 *Mol Biochem Parasitol 48*: 211-222, C Diaz et al. 1992 *Am J Trop Med Hyg 46*: 616-623) and synthetic peptides (U Vergara et al. 1992 *Am J Trop Med Hyg 46*: 39-43) have also been used, again with limited success.

In a multicenter study, the sensitivity and specificity of 10 recombinant antigens used in the analysis with 50 serum samples ranged from 0.95 to 1 and 0.86 to 1, respectively (AO Luquetti 1990 *Mem Inst Oswaldo Cruz 85:* 497-505, A Moncayo & AO Luquetti 1990 *Mem Inst Oswaldo Cruz 85*:

This work was supported in part by PAPES-FIOCRUZ.
+CAPES fellowship and corresponding author. Fax: +55-31-295.3115
++PIBIC-CNPq research fellows
Received 19 February 1997
Accepted 30 July 1997

487-495). However, only three sera from mucocutaneous leishmaniasis and six from visceral leishmaniasis were tested.

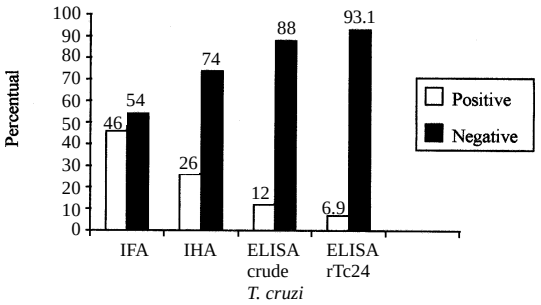
Since double infections may occur (MG Chiaramonte et al. 1996 *Am J Trop Med Hyg 54*: 271-273), specially in our region, the objective of our study was to compare different serological tests used for Chagas' disease among a large number of patients with a well characterized clinic of american cutaneous leishmaniasis. We used IFA, IHA and ELISA with crude antigen and ELISA with a specific *T. cruzi* antigen, a recombinant protein of 24 kDa (rTc24), which was expressed in *Escherichia coli* as a fusion polypeptide with the 26 kDa *Schistosoma japonicum* glutatione S-transferase (GST) (A Ouaissi et al. 1992 *Biol Cell 75*: 11-17).

Our interest in testing this protein resulted from a preliminary data which demonstrated its ability to identify sera of patients with Chagas' disesae, without cross-reactivity with leishmaniasis. However, only few sera were tested (A Taibi et al. 1995 *Parasitology 111*: 581-90). We recently described the ability of rTc24 to distinguish the majority of treated chagasic patients considered non-cured from cured patients (GM Krautz et al. 1995 *J Clin Microbiol 33*: 2086-2090). In the latter group the immunofluorescence tests persisted positive several years after cure (AU Krettli 1984 *Mem Inst Oswaldo Cruz 79*: 59-65, LMC Galvão 1993 *Trans R Soc Trop Med Hyg 87:* 220-223). Among 35 non-chagasic control subjects who had at least three negative serologic results for *T. cruzi*, all were negative in the ELISA using rTc24. Thus, rTc24 seems to be recognized specifically by antibodies anti-*T. cruzi* in sera from chagasic patients with ongoing infections only.

From 1989 to 1995, sera were obtained from 335 patients from the casuistic of the outpatient clinic at Centro de Pesquisas René Rachou, a reference ambulatory for leishmaniasis in the Metropolitan Region of Belo Horizonte. The clinical diagnosis of either mucocutaneous (MCL) or cutaneous leishmaniasis (CL) has been defined both by an active lesion and at least one positive laboratorial exam (Montenegro skin test, immunofluorescence or Giemsa-stained smears from skin biopsies with amastigotes). The patients were included in the study only after written informed consent was obtained from the patient, parent or guardian, according to the official FIOCRUZ ethics committee rules (Ministry of Health). All patients were offered the standard antimonial therapy and presented regression of the lesions. We have not included sera from patients with visceral leishmaniasis since these disease was absent from our sample at the time of the study.

All sera were collected, aliquoted and stored at -20°C. Serology to Chagas' disease was performed by IFA with a cut-off point of 1:20, IHA with a cut-off point of 1:16 and ELISA with crude *T. cruzi* antigen. A standard ELISA was used to detect antibodies against rTc24 protein, with a cut-off point of 0.100, as described (GM Krautz et al. 1995 *J Clin Microbiol 33*: 2086- 2090).

The results of the four different serological tests are shown in Fig. We observed 46% of positive reactions by IFA; 26% by IHA and 12% by ELISA. When the rTc24 was used in ELISA, serum antibodies of 23 patients (6.9%), out of the 335 MCL or CL patients showed significant reactivity. The absorbance values ranged from 0.100 to 0.840 (x=0.248 + 0.161). These 23 patients, 16 men and 7 women, were born and presently live in the State of Minas Gerais; 8 were 6-20 years-old and 15 were ≥ 28 years-old (mean age of 33.4 + 17.96); 21 had the cutaneous form and 2 the mucocutaneous form of ACL. None of them had previous report of Chagas' disease.



Seropositivity to Chagas' disease from 335 patients with american cutaneous leishmaniasis.

The low number of positive reactivity by ELISA using rTc24 among our patients with MCL/CL is compatible with the low prevalence (2-4.5%) of the Chagas' disease in blood donors aged 18-60 years in Minas Gerais (ED Gontijo et al. 1994 *Rev Soc Bras Med Trop 27* Suppl. II: 119-120). The antibodies in sera of individuals above 28 years old may thus be attributed to a higher prevalence of Chagas' disease in Brazil, before the implementation of control measures. A serological survey in school children (7-14 years old) of rural areas was carried out in rural areas of Minas Gerais by the Brazilian National Health Foundation, between 1989 and 1995. When the positive sera were distributed by place of birth in Minas Gerais, we observed that the majority of patients came from areas with prevalence of 2.4 to 11.4/1.000 inhabitants. However, false positive results with rTc24-

ELISA can not be excluded at present. It is unlikely that five of the patients born and resident in the Metropolitan Region of Belo Horizonte have Chagas' disease.

Among the 23 leishmaniasis patients with positive rTc24-ELISA, positive values for anti-*T. cruzi* antibodies were found in 61% by IFA, 56% by IHA and 26% by ELISA using crude antigen. These higher percentage of anti-*T. cruzi* antibodies, when compared to the total percentage of positivity, is expected in case of such patients having double infections. Furthermore, out of the 23 sera, four (17%) were positive in three tests, six (26%) in two and nine (39%) in only one test. Surprisingly, four sera (1.2%) were negative in all three reactions using crude antigen, but were positive by rTc24-ELISA at absorbance values of 0.138, 0.139, 0.198 and 0.281. This fact could be explained by the higher sensibility of the ELISA with recombinant antigen when compared to the other reactions or even due to cross-reactions of the recombinant antigen with other Trypanosomatidae, like *T. rangeli* (Taibi et al. *loc. cit.*). Such patients may have a Chagas infection still non-diagnosed and hemocultures and/or PCR with their blood are like to clarify this possibility.

The use of a purified and specific antigen for *T. cruzi* is a good tool for a reliable serological diagnosis of Chagas' disease in patients with a history of leishmaniasis. However, it is unlikely that all of these 23 patients have double infections. As the only definitive diagnosis of *T. cruzi* infection is actually finding the parasite and/or parasite DNA, hemoculture and/or PCR tests of these 23 patients need to be performed to clarify whether they are indeed chagasic patients with a chronic assymptomatic infection. Alternatively, it may be that rTc24 has some low reactivity with leishmaniasis. Only the careful follow-up of these 23 positive cases will give the correct answer.

Acknowledgements: to Mrs Alda Lima Falcão, Research Scientist, Centro de Pesquisas René Rachou-FIOCRUZ, for providing facilities to work. To Maria Inês Mascarenhas for help with the manuscript editing.