

trans-Sialidase Inhibition Assay, a Highly Sensitive and Specific Diagnostic Test for Chagas' Disease

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For the diagnosis of Chagas' disease, the *trans*-sialidase inhibition assay was able to resolve the results for samples with borderline results, to detect as positive 60% of samples that were negative by conventional serology, and to discriminate idiopathic from chagasic megaviscera or cardiopathy. No cross-reaction with sera from patients with other diseases was observed.

Trypanosoma cruzi is the agent of Chagas' disease, an endemic illness in Latin America. The *trans*-sialidase activity present in *T. cruzi* (10) has not been detected in *Trypanosoma rangeli*, *Leishmania* spp., or *Plasmodium* spp., parasites that share geographical distributions with *T. cruzi*, or in mammals.

We have previously communicated that sera from chronically infected patients display high levels of neutralizing activity against *trans*-sialidase, while sera from patients in the acute phase have lower and variable titers, depending on the time of the evolution of infection (6, 9). These antibodies are transferred via the placenta, but in the absence of congenital infection, titers decrease with the time after birth. In longitudinal follow-up of patients treated during the acute phase and cured after chemotherapy treatment (under the accepted criteria of parasitological cure [12]), a persistence of neutralizing antibodies even 14 years after treatment was found (7). When serum samples from Amerindians who were at high risk of infection and who were negative by conventional serology (CS) and parasitologic assays (hemoculture and xenodiagnosis) were analyzed, they showed neutralizing values similar to those obtained for serum from patients with chronic Chagas' disease (8). These findings might indicate that neutralizing antibodies detected by the *trans*-sialidase inhibition assay (TIA) could identify infections that are undetectable by CS. TIA uses a recombinant *trans*-sialidase (2, 3) that is preincubated with the serum to be tested, and the remnant ability to transfer the sialyl residues from sialyllactose to [D-glucose-1-¹⁴C]lactose is evaluated (5). Pooled normal human sera were used as negative controls, and the value of inhibition obtained was taken as 0% with the cutoff value set at 50% inhibition (6, 7).

The CS means of diagnosis of *T. cruzi* infections, indirect immunofluorescence (IIF) assay and enzyme-linked immunosorbent assay (ELISA), use raw preparations of the parasite, although different defined antigens have been used (4). PCR assay has shown similar or even less sensitivity than serology (1,

11). Since TIA seems to be a highly sensitive diagnostic technique, it was used to analyze a panel of serum samples with different degrees of diagnostic difficulty. Every serum sample in this panel was previously analyzed at least by IIF assay and ELISA.

The panel included the following serum samples. (i) Sera were obtained from Amerindians from Paraguay to increase the number of samples for which results were reported previously (8). Amerindians are at high risk of infection and reside in shacks with elevated number of infected vector bugs. However, a high percentage of this population is parasitologically and serologically negative by conventional assays. Among 73 samples that were analyzed, 24 were simultaneously positive for *T. cruzi* by CS and TIA. Among the 32 serum samples negative by CS, 19 (60%) were reactive by TIA. When samples with borderline results by CS (group I; $n = 17$) were analyzed, 16 (94%) were found to be positive by TIA (Fig. 1).

(ii) Another set of serum samples ($n = 19$) from regions of Paraguay endemic for Chagas' disease with borderline results by CS was included (group II). Among the samples in this group, 53% ($n = 10$) were positive by TIA (Fig. 1).

Serum samples with borderline results (groups I and II) were those with low antibody titers, as detected by IIF assay and ELISA.

(iii) Sera were obtained from patients with idiopathic megaesophagus or megacolon ($n = 38$) or cardiopathy ($n = 4$) from regions of Brazil and Paraguay where Chagas' disease is endemic. Two positive samples were detected by TIA: one from a patient (from Paraguay) with idiopathic megacolon and another obtained from a patient (from Brazil) with idiopathic cardiopathy with a complete bundle branch block as determined by electrocardiography (Fig. 1).

The results described for the first three groups of serum samples (i to iii) show the high sensitivity of TIA (Fig. 1).

To further analyze cross-reactivity with overlapping endemic infections or interference with other disorders, we also studied the following serum samples. (iv) Sera were obtained from patients from Paraguay with cutaneous leishmaniasis who were serologically and parasitologically positive. Among the first group (Fig. 2) of serum samples serologically negative by CS

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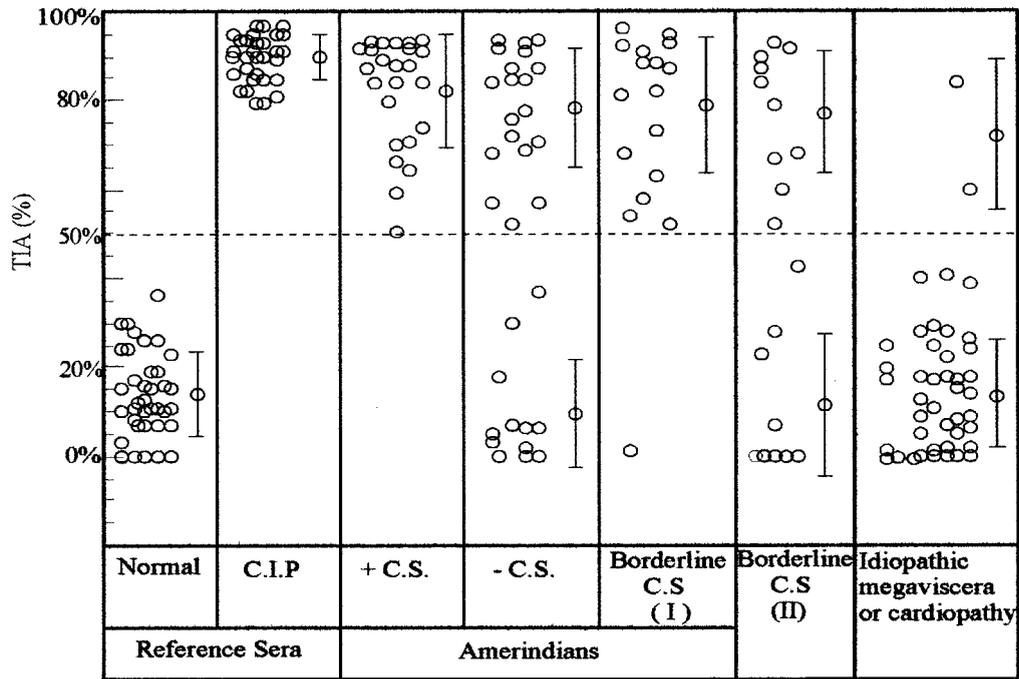


FIG. 1. Sensitivity of TIA. TIA values are for sera from Amerindians from Paraguay that were positive, negative, and borderline by CS (group I) and samples from regions of endemicity in Paraguay with borderline results by CS (group II) and patients with idiopathic cardiopathy or megaviscera. TIA values for sera from healthy individuals and chronically infected patients (CIP) obtained from blood banks in Argentina (negative and positive control groups, respectively) are shown as a reference. Pooled normal human serum from a region of non-endemicity was used to assign 0% inhibition. Cutoff values were set at 50% inhibition. The mean \pm standard deviation is indicated for each group.

for *T. cruzi* ($n = 49$), every serum sample was TIA negative. Among the second group of serum samples ($n = 16$) (Fig. 2) from patients with suspected mixed infection (diagnosed by CS to be positive for *T. cruzi*), only seven samples were reactive by

TIA. The remaining nine samples that were unreactive by TIA coincidentally displayed a low level but positive reactivity for *T. cruzi* by CS. In addition, (v) sera from patients with malaria ($n = 8$) from Brazil, (vi) sera from patients with syphilis ($n =$

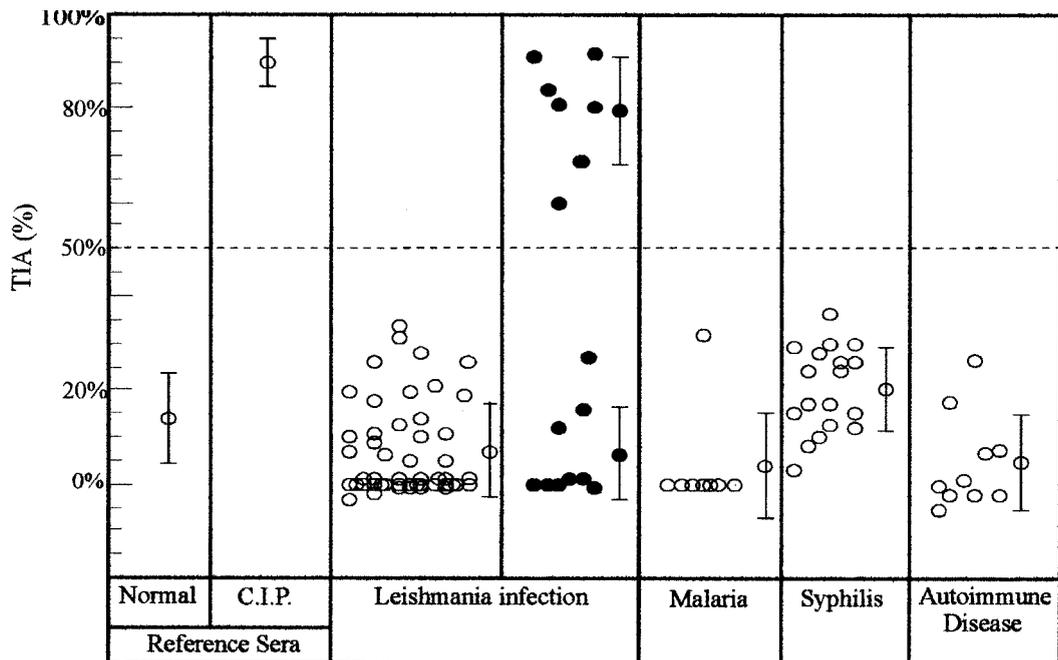


FIG. 2. Specificity of TIA. TIA values are for sera from patients with cutaneous leishmaniasis, malaria, syphilis, and autoimmune diseases that were negative by CS assays (open circles). Solid circles represents TIA values for sera from patients with cutaneous leishmaniasis and suspected simultaneous *T. cruzi* infection as determined by CS. Reference sera were from healthy individuals and chronically infected patients (CIP), as described in the legend to Fig. 1. The mean \pm standard deviation is indicated for each group.

18) from Paraguay, and (vii) sera from patients with autoimmune disease (systemic lupus erythematosus, $n = 6$); rheumatoid arthritis, $n = 1$; pigmentary scleroderma, $n = 1$; myositis, $n = 1$; mixed connective tissue disease, ($n = 1$) from regions of Argentina not endemic for Chagas' disease were also tested.

Serum samples from patients suffering from malaria, syphilis, or autoimmune disease were negative for *T. cruzi* antigens by CS as well as by TIA (Fig. 2). Among the group of patients with cutaneous leishmaniasis, those who were negative by *T. cruzi*-specific CS were also unreactive by TIA. However, among those who were positive by *T. cruzi*-specific CS, seven were found to be TIA positive and nine were found to be TIA negative. Therefore, the low level of reactivity to *T. cruzi* could be due to cross-reactions with epitopes from *Leishmania* sp. antigens. These results demonstrate the specificity of TIA (Fig. 2).

Taken together with the results presented in our previous reports, the results presented here strongly support the view that TIA is a highly specific and sensitive technique for the serological diagnosis of *T. cruzi* infection. It lacks cross-reactivity with other infections like leishmaniasis, malaria, or syphilis, as well as with autoimmune disorders. Its specificity is especially relevant to the solution of the diagnosis problem for those parasitic diseases that have epidemiological distributions similar to those of *T. cruzi* and that carry cross-reactive antigens, like *Leishmania* spp. It is usually hard to discriminate by CS *T. cruzi* and *Leishmania* spp. co-infections. TIA allowed discrimination of those patients infected with both parasites from those whose positivity for *T. cruzi* by CS was due to cross-reactive epitopes from *Leishmania* spp. TIA was also able to resolve samples with borderline results, to detect as positive 60% of CS-negative samples, and to discriminate between idiopathic and infectious megaviscera or cardiopathy.

We propose that use of TIA would be of special interest in blood banks to confirm the results for negative donors and in public health laboratories to confirm or exclude infections in patients.

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