Multicentre evaluation of an antigen-detection ELISA for the diagnosis of *Trypanosoma brucei rhodesiense* sleeping sickness

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The performance of an enzyme-linked immunosorbent assay (antigen ELISA) for the detection, in serum or cerebrospinal fluid, of an invariant trypanosome antigen to diagnose *Trypanosoma brucei rhodesiense* sleeping sickness was evaluated in four clinical treatment centres. The test, which was carried out in polystyrene test-tubes, was positive in 88 (88.9%) of 99 parasitologically confirmed cases that were tested at the National Institute for Medical Research, Tabora, United Republic of Tanzania; 99 (94.3%) of 105 cases tested at the National Sleeping Sickness Control Programme, Jinja, Uganda; 86 (87.8%) of 98 cases tested at the Uganda Trypanosomiasis Research Organisation, Tororo, Uganda; and 59 (96.7%) of 61 cases tested at the Tropical Diseases Research Centre, Ndola, Zambia. The overall detection rate was 91.5%. There was no cross-reactivity with the agents of the common bacterial, viral, or parasitic diseases prevalent in the areas where the studies were conducted. The only false-positive result involved a blood donor from a trypanosomiasis endemic focus. The test was simple to perform, was read visually, and is therefore a potential tool for diagnosing human African trypanosomiasis.

Introduction

Human African trypanosomiasis is one of the major diseases that afflicts the continent. There are two forms of the disease: the chronic variety encountered in West and Central Africa, which is caused by *Trypanosoma brucei gambiense*, and the more acute form, which occurs in East Africa and is caused by *T. b. rhodesiense*. Conservative estimates put the number of new cases diagnosed at 25 000 per annum, with 50 million people at risk in the affected countries in tropical Africa.

Detection of trypanosomes in the blood of an infected individual would be the ideal method for the laboratory diagnosis of African trypanosomiasis. This, however, is not easy, mainly because some of the techniques used have very low sensitivity, while those that are sensitive are not readily applicable in the field (1,2). Several serological techniques have therefore been developed and evaluated extensively in the field as alternatives to the detection of trypanosomes (3,4). Serological tests, however, enable only a presumptive diagnosis to be made, since they cannot determine whether or not a patient has an active infection. Consequently, there is still a need to develop new techniques, particularly those that can distinguish between current and cured infections.⁴,⁵

Recently an approach has been described that involves the detection of trypanosome-invariant antigens in tissue fluids of infected individuals, using an enzyme-linked immunosorbent assay (antigen ELISA) as a means of diagnosis (1). The detection of trypanosome antigens in host tissue fluids would provide evidence of an active infection. The assay, which is based on a monoclonal antibody raised against an invariant antigen of *T. b. rhodesiense* procyclic trypanomastigotes (5), was evaluated in the

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Reprint No. 5247

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laboratory using sera from patients with *T. b. rhodesiense* infection and was highly sensitive and specific (1). It has also shown promise as a method for the diagnosis of *T. b. gambiense* sleeping sickness (6). The studies described here were conducted from July 1989 to July 1990 at four treatment centres (one in the United Republic of Tanzania, two in Uganda, and one in Zambia) to evaluate this antigen ELISA for the field diagnosis of *T. b. rhodesiense* sleeping sickness.

**Materials and methods**

**Patient selection**

Patients who presented to the study treatment centres with histories indicative of sleeping sickness were investigated to confirm the diagnosis, using the following techniques: thick blood film examination (7); haematocrit centrifugation (8); miniblot antibody-coated anion-exchange chromatography (9); single and double centrifugation for trypanosomes in cerebrospinal fluid (CSF) (10); and CSF cell count and protein determination (11). Serum and CSF samples from parasitologically confirmed cases were stored frozen at −20°C and tested later for trypanosome antigens.

To determine the specificity of the antigen ELISA, random serum samples from patients with common viral, bacterial, and parasitic infections, as well as from blood donors, were screened using it.

**Monoclonal antibody**

Polystyrene tubes were coated with the monoclonal antibody TR7/47.34.16, as described by Nantulya et al. (12).

**Antigen detection**

To detect circulating trypanosome antigens, we performed a simplified sandwich ELISA using monoclonal-antibody-coated polystyrene tubes (12). Briefly, 1-ml polystyrene tubes were filled at 4°C with a solution containing 2.5 μg/ml of purified IgM monoclonal antibody TR7/47.34.16; the tubes were capped and then sent from the International Laboratory for Research on Animal Diseases (ILRAD) to the collaborating centres, where they were stored at 4°C.

Immediately before carrying out the assay, the tube contents were tipped out and 25 drops (ca.700 μl) of a 1:500 dilution of peroxidase-labelled monoclonal antibody TR7/47.34.16 were added to each tube without first washing the tubes. The tubes were re-incubated for 15 minutes, rinsed three times, refilled with the same buffer, and left to stand for 15 minutes. The washing buffer was then decanted and the tubes were rinsed three times before 10 drops per tube (250 μl) of substrate (hydrogen peroxide) and chromogen (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) were added to each tube. The tubes were incubated at room temperature with intermittent mixing and the colour changes observed after 30 minutes. For each assay, negative and positive serum controls were included. Negative reactions produced no colour change, while positive reactions resulted in a clear green solution, which made it easy to distinguish visually between positive and negative results.

The assay for antigens in CSF was performed as described for serum except that the CSF (10–20 drops) was tested without prior dilution.

**Results**

**Specificity of the antigen ELISA**

To assess the specificity of the antigen ELISA, we analysed sera obtained from patients with various other infections that were endemic in the study areas. Table 1 shows the pooled results from the four study centres. No cross-reactivity was observed with 36 samples of sera from malaria patients, 33 samples from cases of tuberculosis, or 17 samples from cases of human immunodeficiency virus (HIV) infection. One sample of serum from a blood donor from an endemic area was weakly positive for antigen; it was, however, not possible to make exhaustive in-

![Table 1: Results of the antigen-ELISA on samples of sera obtained from various categories of patients to assess the specificity of the assay](image)

<table>
<thead>
<tr>
<th>Category of patient</th>
<th>No. tested</th>
<th>No. positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>HIV infection b</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Blood donors</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Syphilis</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Filariasis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>1 (0.9%)</td>
</tr>
</tbody>
</table>

* Pooled results for the four collaborating centres.

b HIV = human immunodeficiency virus.
investigations on this patient to rule out a trypanosome infection. The assay thus demonstrated a high degree of specificity.

Sensitivity of the antigen ELISA

For some patients, trypanosome antigens were detected in serum only; for others, they were detected in CSF only; and for yet others the antigens were detected in both serum and CSF. The pattern for two centres where paired CSF and serum analyses were performed is shown in Table 2. Nearly half the patients (49.4%) tested positive for antigen in serum only; 9.4% in CSF only; and the rest in both serum and CSF. For calculation of the detection rates, we therefore considered patients to have tested positive if their serum and/or CSF was ELISA-positive (Table 3).

A total of 363 parasitologically proven cases were tested using the antigen ELISA in the four study centres. The results are summarized in Table 3. Of these patients, 332 gave positive results, corresponding to an overall sensitivity of 91.5%. At the National Institute for Medical Research (NIMR), United Republic of Tanzania, 99 cases were tested, of which 88 (88.9%) were antigen-positive in the ELISA. At the National Sleeping Sickness Control Programme (NSSCP), Uganda, 99 (94.3%) of 105 cases tested gave positive results, while at the Uganda Trypanosomiasis Research Organisation (UTRO) 86 (87.8%) of the 98 cases tested were positive. At the Tropical Diseases Research Centre (TDRC) Zambia, 59 (96.7%) of 61 patients tested were positive.

A total of 30 patients (8.3%) in the whole series tested negative for antigens (Table 3).

Analysis of CSF results

A comparison between the results of the antigen ELISA for CSF and other changes in CSF, such as the presence of trypanosomes, increased protein levels, and elevated white cell counts, is given in Table 4 and Table 5. Trypanosomes were detected in the CSF of 66.7% of the patients whose CSF gave a positive antigen ELISA result; the protein levels were elevated in 48.7%; and the white cell counts were elevated in 87.2% (Table 4). Conversely, 70.4% of patients whose CSF contained trypanosomes (Table 5) had detectable levels of trypanosome antigens in their CSF; 65.5% had elevated protein levels; and 96.3% had elevated white cell counts.

### Antigen-detection ELISA for diagnosing human African Trypanosomiasis

<table>
<thead>
<tr>
<th>Study centre</th>
<th>No. of cases tested</th>
<th>No. of cases with positive antigen ELISA in serum</th>
<th>No. of cases with positive antigen ELISA in CSF</th>
<th>Total number of cases with positive antigen ELISA in serum and/or CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIMR</td>
<td>99</td>
<td>88 (88.9)</td>
<td>N.A.</td>
<td>88 (88.9)</td>
</tr>
<tr>
<td>NSSCP</td>
<td>105</td>
<td>89 (84.8)</td>
<td>10 (9.5)</td>
<td>99 (94.3)</td>
</tr>
<tr>
<td>UTRO</td>
<td>98</td>
<td>79 (80.6)</td>
<td>7 (7.1)</td>
<td>86 (87.8)</td>
</tr>
<tr>
<td>TDRC</td>
<td>61</td>
<td>59 (96.7)</td>
<td>N.A.</td>
<td>59 (96.7)</td>
</tr>
<tr>
<td>Total</td>
<td>363</td>
<td>314 (86.5)</td>
<td>18 (5.0)</td>
<td>332 (91.5)</td>
</tr>
</tbody>
</table>

**Notes:**
- NIMR = National Institute for Medical Research, Tabora, United Republic of Tanzania; NSSCP = National Sleeping Sickness Control Programme, Jinja, Uganda; UTRO = Uganda Trypanosomiasis Research Organisation, Tororo; TDRC = Tropical Diseases Research Centre, Ndola, Zambia.
- Figures in parentheses are percentages.
- N.A. = not available.

Table 3: Ability of the antigen ELISA to detect trypanosome antigens in samples of sera from patients with parasitologically proven Trypanosoma brucei rhodesiense sleeping sickness

Table 2: Pattern for the detection of trypanosome antigens in samples of serum and cerebrospinal fluid (CSF) of Trypanosoma brucei rhodesiense patients

<table>
<thead>
<tr>
<th>Study centre</th>
<th>No. of cases antigen-ELISA-positive in:</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>CSF</td>
</tr>
<tr>
<td>UTRO</td>
<td>34</td>
<td>7</td>
</tr>
<tr>
<td>NSSCP</td>
<td>55</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>89 (49.4)c</td>
<td>17 (9.4)</td>
</tr>
</tbody>
</table>

* UTRO = Uganda Trypanosomiasis Research Organisation, Tororo; NSSCP = National Sleeping Sickness Control Programme, Jinja, Uganda.
* Five other patients in the series were excluded from the analyses because they did not have paired CSF and serum results.
* Figures in parentheses are percentages.
Table 4: White cell counts, protein levels, and presence of trypanosomes in cerebrospinal fluid (CSF) of patients with Trypanosoma brucei rhodesiense sleeping sickness whose CSF was positive in the antigen ELISA

<table>
<thead>
<tr>
<th>CSF parameter</th>
<th>No. tested</th>
<th>No. positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of trypanosomes</td>
<td>57</td>
<td>38 (66.7)^b</td>
</tr>
<tr>
<td>Elevated cell count</td>
<td>86</td>
<td>75 (87.2)</td>
</tr>
<tr>
<td>Elevated protein level</td>
<td>39</td>
<td>19 (48.7)</td>
</tr>
</tbody>
</table>

^a Pooled results for the Uganda Trypanosomiasis Research Organisation (UTRO) and the National Sleeping Sickness Control Programme (NSSCP).
^b Figures in parentheses are percentages.

Table 5: Antigen ELISA results, white cell counts, and protein levels in the cerebrospinal fluid (CSF) of patients with Trypanosoma brucei rhodesiense whose CSF contained trypanosomes

<table>
<thead>
<tr>
<th></th>
<th>No. tested</th>
<th>No. positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen ELISA</td>
<td>54</td>
<td>38 (70.4)^b</td>
</tr>
<tr>
<td>Elevated cell count</td>
<td>54</td>
<td>52 (96.3)</td>
</tr>
<tr>
<td>Elevated protein level</td>
<td>29</td>
<td>19 (65.5)</td>
</tr>
</tbody>
</table>

^a Pooled results from the Uganda Trypanosomiasis Research Organisation (UTRO) and National Sleeping Sickness Control Programme (NSSCP).
^b Figures in parentheses are percentages.

Discussion

In the study we have described, an antigen-trapping ELISA (13) was evaluated in four treatment centres for the diagnosis of T. b. rhodesiense infection. The detection rate varied from 87.8% to 96.7%, and the overall detection rate was 91.5%, which is in agreement with that previously reported for an assessment of the same monoclonal antibody in a microplate ELISA (1). This level of sensitivity also compared favourably with that obtained using anti-trypanosome antibody detection techniques, such as the indirect immunofluorescent antibody test (13), ELISA (14), card agglutination (4), and procyclic agglutination (15) tests. Whereas antibody assays can provide only a presumptive diagnosis, demonstration of specific trypanosome antigens in the tissue fluids of a patient is evidence of a current infection. Antigen positivity is thus synonymous with parasitological diagnosis, and hence provides a more direct basis for initiation of chemotherapy.

Cross-reactivity was not observed with other diseases that are endemic in the study areas, such as malaria, schistosomiasis, tuberculosis, syphilis, filariasis, and acquired immunodeficiency syndrome (AIDS). Only one blood donor from an endemic area gave a false positive reaction. The assay thus appears to be specific and this represents an improvement over the other immunodiagnostic tests, which on average give false positivity rates of 5% (14,16).

Despite its high sensitivity, the test nevertheless failed to detect a total of 30 (8.3%) out of 363 parasitologically diagnosed cases from the four collaborating centres. These false negative cases probably represent a group at an early stage (less than 2 weeks) of infection, whose circulating antigens were below the assay's limit of detection (1,7,17). However, these antigen-ELISA-negative patients were readily detected by parasitological techniques, which indicates that antigen detection and parasitological diagnosis are complementary.

Because in experimental infections in animals, antigen levels fluctuate throughout the course of infection (17,18), the assay should be repeated at least once on patients with a highly indicative clinical history but who do not have demonstrable parasitaemia.

With some patients, antigens were detected in CSF but not in serum. Apart from the diagnostic implication of this, i.e., that a proper diagnostic investigation of a patient should include a search for trypanosome antigens in both CSF and serum, this finding emphasizes the independent nature of the blood and CSF compartments, as has also been observed for the distribution of anti-trypanosome antibodies in the infected host (19).

There was a strong correlation between the presence of antigens in CSF and other pathological changes associated with involvement of the central nervous system, i.e., an increase in protein levels and white cell counts, and the presence of trypanosomes in the CSF compartment. A similar correlation was observed in T. b. gambiense infections, where the antigen ELISA has major diagnostic potential (7). These results suggest that, apart from its use as a diagnostic tool, the assay can be interpreted alongside existing criteria for clinically staging the disease. Another potential application of the assay is for evaluating the success of treatment (7,17), and this is currently being investigated.

Acknowledgements

This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) (Grant No. ID 890041). We wish to thank all the individuals who assisted in collecting the samples and managing patients, Mr J.T. Njuguna for technical assistance, and Mrs Risper Okonji for secretarial assistance.
Résumé

Evaluation multicentrique d'un ELISA pour la recherche de l'antigène servant au diagnostic de la trypanosomiase à Trypanosoma brucei rhodesiense

On a évalué dans quatre centres de traitement clinique les résultats d'une réaction immunoenzymatique (ELISA-antigène) visant à mettre en évidence dans le sérum ou le liquide céphalorachidien un antigène trypanosomique invariant, qui permettrait de diagnostiquer une trypanosomiase à Trypanosoma brucei rhodesiense. Ce test, qui a été effectué dans des tubes à essai en polystyrene, a été positif pour 88 (88,9%) des 99 cas parasitologiquement confirmés testés au National Institute for Medical Research de Tabora, République-Unie de Tanzanie, pour 99 (94,3%) des 105 cas testés dans le cadre du National Sleeping Control Program de Jinja, Ouganda, pour 86 (87,8%) des 98 cas testés par l'Uganda Trypanosomiasis Research Organisation de Tororo, Ouganda, et pour 59 (96,7) des 61 cas testés au Tropical Diseases Research Centre de Ndola, Zambie. Le taux global de mise en évidence a été de 91,5%. Il n'y a pas eu de réactivité croisée avec les germes des maladies bactériennes, virales ou parasitaires répandues dans ces régions. Le seul faux positif obtenu était dû à un donneur de sang provenant d'un foyer d'endémie de la trypanosomiase. Ce test est d'exécution simple, la lecture du résultat est visuelle et il constitue par conséquent un outil potentiellement utile pour le diagnostic de la trypanosomiase africaine chez l'homme.

References