

Diagnosing human African trypanosomiasis in Angola using a card agglutination test: observational study of active and passive case finding strategies

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Abstract

Objective To assess the operational feasibility of detecting human African trypanosomiasis by active and passive case finding using the card agglutination test with serial dilution of serum to guide treatment. **Setting** Trypanosomiasis control programme in the Negage focus, northern Angola, during a period of civil war.

Design Observational study.

Participants 359 patients presenting themselves to health centres with symptoms (passive case finding) and 14 446 people actively screened in villages.

Main outcome measures Whole blood and serological tests at different dilutions using the card agglutination test, and detection of parasites by microscopy.

Results Active case finding identified 251 people with a positive card agglutination test result, 10 of whom had confirmed parasites. In those presenting for investigation 34 of 51 with a positive card agglutination test result at the dilution of 1:8 or more used to guide treatment had parasites in blood, lymph node fluid, or cerebrospinal fluid compared with 10 of 76 in those detected by active case finding; positive predictive values of 67% for passive case detection and 13% for active case detection. Only at a cut-off dilution of more than 1:32 was the positive predictive value in active case detection reasonable (46%) and at this dilution 40% of microscopically proved cases were missed.

Conclusions The card agglutination test is useful for initial screening in active detection of cases with human African trypanosomiasis but, given the toxicity of the drugs, serology using the card agglutination test should be not used alone to guide treatment after active case finding. A second confirmatory test is needed.

Introduction

Human African trypanosomiasis has two forms, *Trypanosoma brucei gambiense* and *T b rhodesiense*. The disease is most common in areas of current or recent civil breakdown; Angola has around 100 000 cases. *T b gambiense* causes sleeping sickness, the chronic, slowly progressive form of trypanosomiasis.

Early diagnosis and treatment of trypanosomiasis is essential, as late stage disease is associated with high mortality. Early treatment helps to interrupt the parasite's transmission cycle, providing an efficient control strategy for active case finding.¹ However, over-diagnosis and over-treatment are potentially dangerous; the drugs used to treat trypanosomiasis are associated with severe side effects, high costs, and diffi-

culties in administration. Diagnostic systems therefore need to detect cases early and minimise false positives.

The card agglutination test, developed for the detection of *T b gambiense* specific antibodies,² is a simple, cheap, tool for rapid screening of high patient numbers under field conditions using whole blood. The World Health Organization recommends follow-up every 3-6 months for people with a positive test result but no parasites on microscopic examination of blood and lymph node fluid. This is difficult to maintain in areas of complex emergency. Serial dilution of serum before using the card agglutination test has been suggested as a diagnostic confirmatory assay in those who have an initial positive result on screening to avoid prolonged waits.³ Diluting serum, however, increases the specificity at the possible expense of sensitivity; the higher the dilution at which a test remains positive the more likely it is to be a true positive.

During the recent civil war in Angola few areas of the country could be reached easily and a screening approach requiring only one visit was the only realistic way to identify cases and contain the infection reservoir. Between 1999 and 2002 the control programme for trypanosomiasis in Angola decided that all people who had a positive serology result at a serum dilution of 1:8 or more with the card agglutination test should be treated regardless of evidence of parasites on microscopy.⁴

We determined whether titration using the card agglutination test can guide treatment in potential cases of human African trypanosomiasis detected by passive and active case finding.

Participants and methods

The study took place from February to May 2001 in the Negage focus, northern Angola, where the human African trypanosomiasis control programme, Angotrip, is run. The study took place during the final stage of the civil war. Patients with symptoms (see bmj.com) presented themselves to the treatment centre in Negage (passive case finding). Active case finding was by screening all people (about 63% attended) in adjacent villages, irrespective of symptoms.

We consecutively registered all patients from both groups and screened whole blood from each by the card agglutination test (Laboratory of Serology, Institute of Tropical Medicine, Antwerp, Belgium). People with a positive test result underwent cervical lymph node examination for lymphadenopathy and, if

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present, examination of a wet preparation of lymph node fluid (see bmj.com for flow of screening process). Those without palpable lymph nodes or with a negative lymph node fluid result had their blood examined for parasites by capillary tube centrifugation (two tubes for each person).⁵ The cerebrospinal fluid was double centrifuged and searched for trypanosomes.⁶ We used the Fuchs-Rosenthal chamber to count white blood cells in the cerebrospinal fluid.

For people with a positive card agglutination test result but negative microscopic examination of blood and lymph node fluid, we determined the serum end titre using the test. Patients with detectable parasites underwent further blood testing to determine their serological status. For serum titration we centrifuged 5 ml of blood; we tested serum with serial twofold dilution in test buffer solution and determined the highest dilution at which a sample remained positive. We considered people infected who were either positive for parasites or positive using the test at a serum dilution of 1:8 or greater.

We assessed the sensitivity and positive predictive value of serum titres at various cut-off dilutions. The gold standard was microscopically proved cases. We report the relative sensitivities of higher dilutions because the full parasitological investigation was not carried out on all those with a negative test result on whole blood.

Results

We consecutively screened 14 446 people of all ages by active case finding. In total 359 people with symptoms presented themselves to the treatment centre and were consecutively registered in the study (passive case finding).

Active case finding led to the identification of 251 people with a positive card agglutination test result (seroprevalence 1.7%). Seventy six people aged 5-72 years had a positive reaction in serum titration of 1:8 or more, but in only 10 (13%) were parasites detected in

Table 1 Evaluation of card agglutination test compared with parasite proved disease after active case finding of people with human African trypanosomiasis identified by mass screening

Cut-off dilution using card agglutination test	Conventional parasitological methods*		Total
	No with positive result	No with negative result	
Whole blood:			
Positive result	10	241†	251
Negative result	0	14 195	14 195
Total	10	14 436	14 446
≥1:8:			
Positive result	10	66†	76
Negative result	0	14 370	14 370
Total	10	14 436	14 446
≥1:16:			
Positive result	10	39†	49
Negative result	0	14 397	14 397
Total	10	14 436	14 446
≥1:32:			
Positive result	6	7†	13
Negative result	4	14 429	14 433
Total	10	14 436	14 446

*Capillary tube centrifugation, wet examination of lymph fluid, and examination of cerebrospinal fluid.
†False positive result for serology.

Table 2 Evaluation of card agglutination test compared with parasite proved disease using passive case detection where patients have presented with symptoms of trypanosomiasis for diagnosis

Cut-off dilution using card agglutination test	Conventional parasitological methods*		Total
	No with positive result	No with negative result	
Whole blood:			
Positive result	51	32†	83
Negative result	0	276	276
Total	51	308	359
≥1:8:			
Positive result	34	17†	51
Negative result	0	308	308
Total	34	325	359
≥1:16:			
Positive result	33	11†	44
Negative result	1	314	315
Total	34	325	359
≥1:32:			
Positive result	21	2†	23
Negative result	13	323	336
Total	34	325	359

*Capillary tube centrifugation, wet examination of lymph fluid, and examination of cerebrospinal fluid.
†False positive result for serology.

lymph node fluid or blood (table 1). Of these just over half had symptoms (see bmj.com). Parasites were found only in people with test titres of 1:16 or greater. No patients identified by active case finding had detectable parasites in the cerebrospinal fluid. The prevalence of parasitologically proved disease was low: 0.07% (10/14 446).

Eighty three people identified by passive case finding had a positive card agglutination test result (seroprevalence 23.1%). Fifty one people aged 1 year to 56 years had elevated card agglutination test titres, of whom 34 (67%) had detectable parasites (table 2). Parasites were not found in people with test titres less than 1:8, and only one patient with parasitologically proved disease had a titre of 1:8. Haemolymphatic examination detected 65% (22/34) of patients with parasitologically proved disease (see bmj.com). In 12 patients (35% of the total) trypanosomes were found only in the cerebrospinal fluid; six were either asymptomatic or had non-specific symptoms.

A positive card agglutination test result on whole blood using active case finding has a 4% positive predictive value with a ratio of false to true positives of 24:1 (table 3). When the cut-off titre is 1:8, the current threshold to treat, the positive predictive value is 13.2%. At a cut-off dilution greater than 1:32 the positive predictive value is 46% (table 3), but 40% of microscopically proved cases are missed.

When using a passive case finding strategy, the card agglutination test on whole blood has a 61.4% positive predictive value. When the cut-off titre is 1:8, the positive predictive value is 66.7%. When the cut-off dilution is 1:32, the positive predictive value is 91.3%, but 38% of parasitologically proved cases are missed.

Discussion

At the prevalence of human African trypanosomiasis found in the general population (<1%) the positive predictive value of the card agglutination test at the

Table 3 Relative sensitivity and positive and negative predictive values of card agglutination test compared with parasitologically proved trypanosomiasis by active and passive case detection

Cut-off dilution using card agglutination test	Relative sensitivity (%)*	% positive predictive value (95% CI)
Active case detection:		
Whole blood	—	4.0 (2 to 7)
≥1:8	100 (66 to 100)	13.2 (7 to 23)
≥1:16	100 (66 to 100)	20.4 (11 to 35)
≥1:32	60.1 (27 to 86)	46.2 (20 to 74)
Passive case detection:		
Whole blood	100 (91 to 100)	61.4 (50 to 72)
≥1:8	100 (87 to 100)	66.7 (52 to 79)
≥1:16	97.1 (83 to 100)	75.0 (59 to 86)
≥1:32	61.8 (44 to 77)	91.3 (70 to 98)

*Starting population in active case finding of those screening positive on whole blood.

†Not able to determine accurately as asymptomatic people serologically negative in active screening were not investigated further.

conventional serological cut-off dilution of 1:8 is too low (13%) to guide treatment on its own. Given the cost and toxicity of the drugs used to treat trypanosomiasis this is serious; where positive serological tests alone are used to guide treatment the overall effect could even be harmful. The specificity of the card agglutination test is less than 100%, probably because other diseases that induce false positive reactions may cause symptoms. According to our data, when the prevalence of trypanosomiasis is about 10%, only a cut-off dilution of 1:32 in passive surveillance presents reasonable high positive predictive value (91%) to be a sole guide to treatment with available drugs, but at the cost of missing a significant number of true cases.

Although microscopy is virtually 100% specific the card agglutination test has false positives. Malaria, schistosomiasis, filariasis (all prevalent in the area), and toxoplasmosis may induce false positive low titre card agglutination test results. Tsetse flies are generally infected by non-pathogenic trypanosomes at higher rates than by *T b gambiense*. Non-pathogenic trypanosomes could persist in humans long enough to induce a positive test result.

It is possible that newer and more specific serological tests could improve on this, but even the best are not 100% specific under field conditions.^{7,8} We found that for active case finding of trypanosomiasis, serology is likely to be useful in identifying those who need further testing with an unrelated test, currently by conventional parasitological methods.

Carrying out research in complex emergency settings imposes limitations. The chance is that some patients who have a positive card agglutination test result but whose disease is not parasitologically proved would have gone on to have detectable parasites if repeatedly sampled as recommended by WHO. This was not a practical option in Angola at the time of the study.

During the Angolan civil war many areas were not accessible for health workers. The trypanosomiasis control strategy had to be adapted to the reality that a single examination of villagers might be their only chance to access treatment. Case finding is a key part of control. Even in stable situations long delays occur in diagnosis, during which patients will deteriorate, while remaining a reservoir of infection.⁹ The introduction of

What is already known on this topic

To prevent potentially fatal progression of human African trypanosomiasis, and for control of the disease, cases must be diagnosed early

Active case detection using the card agglutination test with treatment given to those with a positive test at 1:8 dilution has been advocated

What this study adds

Using the card agglutination test as the sole guide to treatment of those identified by active case detection leads to substantial over-diagnosis of the disease, with potentially serious consequences

A two stage screening process with a sensitive test for active case detection and a specific test for case confirmation is the most appropriate strategy

titration on the card agglutination test helped to identify many infected patients and possibly to contain the spread of the infection, but also subjected people who were false positive to harmful treatments. Since 2002 Angola has been heading for political stability. Where resources are severely constrained, as they are in most areas where trypanosomiasis is a problem, using two tests where one is adequate has to be avoided as it is costly.¹⁰ Our study, however, suggests that the potential dangers of using laboratory investigations that have even a reasonable specificity as the sole guide for trypanosomiasis treatment where the prevalence is low (all active case finding) are considerable in trypanosomiasis. Treatment should be based on a two stage screening process where the first test is followed by another, independent test in those found positive.

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- Méda HA, Pépin J. The epidemiology and control of human African trypanosomiasis. *Adv Parasitol* 2001;49:71-132.
- Magnus E, Vervoot T, Van Meirvenne N. A card-agglutination test with stained trypanosomes (CATT) for the serological diagnosis of *T b gambiense* trypanosomiasis. *Ann Soc Belg Med Trop* 1978;58:169-76.
- Simarro PP, Ruiz JA, Franco JR, Josenando T. Attitude towards CATT-positive individuals without parasitological confirmation in the African trypanosomiasis (*T gambiense*) focus of Quiçama (Angola). *Trop Med Int Health* 1999;12:858-61.
- Stanghellini A, Josenando T. The situation of sleeping sickness in Angola: a calamity. *Trop Med Int Health* 2001;6:330-4.
- Woo PTK. The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Trop* 1970;27:384-6.
- Cattand P, Miezán BT, De Raadt P. Human African trypanosomiasis: use of double centrifugation of cerebrospinal fluid to detect trypanosomes. *Bull World Health Organ* 1988;66:83-6.
- Papadopoulos MC, Abel PM, Agranoff D, Stich A, Tarelli E, Bell BA, et al. A novel and accurate diagnostic test for human African trypanosomiasis. *Lancet* 2004;363:1358-63.
- Chappuis F, Loutan L, Simarro P, Lejon V, Buscher P. Options for field diagnosis of human African trypanosomiasis. *Clin Microbiol Rev* 2005;18:133-46.
- Odiit M, Shaw A, Welburn SC, Fevre EM, Coleman PG, McDermott JJ. Assessing the patterns of health-seeking behaviour and awareness among sleeping-sickness patients in eastern Uganda. *Ann Trop Med Parasitol* 2004;98:339-48.
- Lutumba P, Robays J, Miaka C, Kande V, Simarro PP, Shaw AP, et al. Efficience de différentes stratégies de détection de la trypanosomiase humaine africaine à *T b gambiense*. *Trop Med Int Health* 2005;10:347-56. (Accepted 24 March 2006)

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