# Detection of trypanosomes in suspected sleeping sickness patients in Uganda using the polymerase chain reaction

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Diagnosis of sleeping sickness (trypanosomiasis) is difficult because of the fluctuating levels of parasitaemia encountered in patients. In the present study we found that the polymerase chain reaction (PCR) demonstrated trypanosome infection in 20 out of 35 (57.1%) blood samples and in 21 out of 34 (61.7%) cerebrospinal fluid (CSF) samples collected from an area endemic for sleeping sickness in north-west Uganda. A total of 14 blood samples and 13 CSF samples that were positive for trypanosomes by double centrifugation were also positive by PCR, demonstrating good concordance between the two methods. However, 6 (28.6%) of the 21 blood samples that were parasitologically negative were positive by PCR, while 8 (38.0%) out of 21 CSF samples that were negative by double centrifugation were positive by PCR. These 14 negative samples could therefore be from sleeping sickness cases even though a positive PCR test is not evidence for the presence of trypanosomes. Furthermore, of these 8 CSF samples, 4 had been designated as early cases, based on the absence of trypanosomes and on a count of $\leqslant 5$ white blood cells (WBC) per μl. This suggests that some late-stage cases could potentially be missed according to the present criteria, and it is therefore important to perform clinical trials to determine whether these cases could be treated successfully with the first-stage drug alone. The remaining four CSF samples had been classified as late-stage cases, based on a count of >6 WBC per μl, even though trypanosomes could not be detected in these samples by either double centrifugation or PCR. A cut-off point of 5 WBC per μl, which is used as a rule of thumb to stage sleeping sickness patients, seems to leave some late-stage cases undetected since trypanosomes were detected in four CSF samples from suspected cases with <5 WBC per μl.

**Keywords:** agglutination tests; centrifugation; hematocrit; polymerase chain reaction; trypanosomiasis, African; Uganda.

*Voir page 123 le résumé en français. En la página 124 figura un resumen en español.*

## Introduction

In Uganda, human African trypanosomiasis (sleeping sickness) is caused by two different trypanosome subspecies — *Trypanosoma brucei rhodesiense* (which causes the acute form of sleeping sickness in the south-east of the country), and *T.b. gambiense* (which causes the chronic form of sleeping sickness in the north-east and north-west). Epidemics of Gambian sleeping sickness, which have occurred periodically in north-east and north-west Uganda since 1983, have been attributed partly to population movements from the Sudan owing to the civil and political unrest there. The disease is fatal if untreated. Since most drugs currently available for treatment of sleeping sickness are very toxic (*1*), the diagnosis must be reliable. Various methods are at present used for diagnosis, including serological tests for specific antibodies, such as the card agglutination test for trypanosomiasis (CATT: Testryp-CATT; Smith-Kline-RIT, Antwerp, Belgium) (*2*). These tests are useful for mass screening, but do not discriminate between current and past infections (*1*). Parasitological diagnostic methods are more accurate, but have low sensitivity and may not give reproducible results, especially with the characteristically low parasitaemias in Gambian sleeping sickness which often remain undetected when they are used.

Techniques based on the amplification and characterization of nucleic acids from infectious organisms are sensitive and rapid (*3, 4*). Polymerase chain reaction (PCR) amplification has been advocated for the molecular diagnosis of several genetic diseases (*5, 6*) and for the identification of infectious disease agents, including trypanosomes (*7, 8*) and human immunodeficiency virus (*9, 10*).

In PCR diagnosis of trypanosome infections, the target DNA may be repetitive DNA, ribosomal RNA genes, or kinetoplast DNA minicircles. Since multiple copies of all these are present, the sensitivity is very high (*3*). In order to establish the stage of the

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disease, a PCR test with primers specific to *T. brucei* species (*7, 8*) was used in the present study to detect trypanosomes in the blood and cerebrospinal fluid from suspected sleeping sickness patients in Uganda.

## Materials and methods

### Field study and collection of samples

From November 1997 to January 1998, surveys were carried out in areas with endemic sleeping sickness in Arua district, north-west Uganda, by staff of the Livestock Health Research Institute (LIRI), Tororo. During active surveillance, persons were mobilized by sleeping sickness health aides and local chiefs to assemble at selected screening centres for examination using CATT as a primary screening test. Blood samples from suspected cases who were CATT-positive were then examined by thick blood smears (TBS) and the haematocrit centrifugation technique (HCT) for the presence of trypanosomes. The miniature anion-exchange centrifugation technique (MAECT) was used on suspected cases who were CATT-positive, but negative by TBS examination and HCT. If the clinical signs and a positive CATT indicated infection, but the blood was aparasitaemic, a lumbar puncture was performed. In each case the cerebrospinal fluid (CSF) was examined for the presence of trypanosomes after double centrifugation, and the cells were counted.

Blood samples from each suspected patient who gave a strong agglutination reaction (+++ or ++ in the CATT), or in whom parasites were detected, were collected into two microcentrifuge tubes with 10 mmol/l ethylenediaminetetraacetic acid (EDTA) for PCR (two 0.5-ml samples). The CSF samples from parasitologically confirmed cases and suspected cases, based on clinical signs, were also collected in microcentrifuge tubes for PCR (two 0.5-ml samples). In each case, the samples were stored in liquid nitrogen prior to transportation to the LIRI laboratory in Tororo.

### Extraction of DNA for PCR

A combined lysis–phenol extraction method was used to prepare DNA from the blood or CSF samples, as described by Van der Ploeg et al. (*11*). Briefly, 0.5-ml samples of blood or CSF from each suspected case were mixed with lysis buffer (1% NP-40 in 10 mmol/l Tris-HCl, pH 7.5) in a microcentrifuge tube. The mixture was centrifuged at 10 000 *g* for 1 min, the supernatant removed, and the pellet resuspended in 1 ml of the same buffer and mixed by vortexing. The procedure was repeated with the same buffer to wash the DNA extract. The extract was then centrifuged at 10 000 *g* for 1 min and the supernatant discarded. The pellet was resuspended in 0.2 ml of SE buffer (10 mmol/l Tris-HCl, 100 mmol/l sodium chloride, 100 mmol/l ethylenediaminetetraacetic acid, pH 8.0) containing 2% *N*-laurylsarconine, sodium salt, with proteinase K (EC 3.4.21.64) at 100 µg/ml. Samples were incubated at 37 °C for 30 min and each sample was extracted three times with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1 by volume). The DNA was precipitated by adding 0.1 vol. of 3 mol/l sodium acetate, pH 5.2, and 0.6 vol. of absolute ethanol, and incubated at –20 °C for 30 min. The precipitated DNA was centrifuged at 10 000 *g* for 1 min and the resultant pellet washed with 70% ethanol. The clean DNA was air-dried, resuspended in 100 µl distilled de-ionized water, and kept at 4 °C until use.

### Polymerase chain reaction (PCR)

The PCR method used was essentially that described by Masiga et al. (*8*). Two PCR primers, both specific for *T. brucei* subspecies (*8*) were used: $TBR_1$ (5′-GAATATTAAACAATGCGCAG-3′) and $TBR_2$ (5′-CCATTTATTAGCTTTGTTCG-3′). PCR amplifications were carried out in 50 µl reaction mixtures containing, as final concentrations, 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l potassium chloride, 1.5 mmol/l magnesium chloride, 200 µmol each of the four deoxynucleoside triphosphates (dNTPs), primers at 1 µmol/l each, 1.5 U of Red Hot DNA polymerase (Advanced Biotechnologies, NY, USA; Lot: RO2HO6-3, Cat. No. ab04006/B) and the DNA template. 10 ng genomic DNA of *T.b. gambiense* Dal 972 was used in the positive control tubes, while a tube containing no DNA was used as a negative control for each set of amplification reactions. Various amounts of DNA preparations were tried and 15 µl gave the best bands; this amount was then used routinely. Each reaction mixture was overlaid with a drop of paraffin oil to prevent evaporation, and amplified in a Hybaid thermal cycler with the initial denaturation step at 94 °C, annealing at 50 °C for 2 min, and extension at 72 °C for 1 min. At the end of 30 cycles, the samples were cooled slowly to room temperature over a minimum of 10 min. Each DNA sample was amplified twice to check the reproducibility of the results. Finally, 15 µl of each amplified sample was analysed by electrophoresis in 1.2% agarose gel containing ethidium bromide (0.5 µg/ml). The amplified products were visualized by ultraviolet transillumination.

### Statistical analysis

Fisher's exact test for $2 \times 2$ tables (1990 Graph Pad Instat software, version 1.11a) and the Pearson's $\chi^2$ test were used to analyse the results.

## Results

### Field survey

A total of 556 persons were examined by a combination of CATT, TBS examination, and HCT. A total of 113 (20.3%) of the 556 persons screened by CATT using whole blood were found to be CATT positive. Further CATT testing of these positive cases was carried out using plasma diluted

1/4 and 1/8 to reduce the number of CATT-positive samples. MAECT was used for cases that were highly suspected on clinical grounds who were positive by CATT, but negative by both HCT and TBS examination.

## Detection of trypanosomes in suspected sleeping sickness patients

Parasitological examination and PCR amplification were carried out on a total of 35 blood samples (33 CATT-positive and 2 CATT-negative) using specific primers for *T. brucei* subspecies to demonstrate the absence or presence of trypanosomes. Each sample was examined twice by PCR before the results were confirmed. A total of 20 (60.6%) of the 33 CATT-positive blood samples were positive by PCR, while 13 (39.4%) were PCR- negative (Table 1). In addition, two blood samples that were negative by CATT were also negative by PCR. However, trypanosomes were demonstrated parasitologically in 14 (70%) of the 20 blood samples which were positive by both CATT and PCR (Table 1).

Similarly, 34 CSF samples from a total of 556 persons were examined for the presence of trypanosomes by double centrifugation and PCR techniques. When the cut-off point for early-stage sleeping sickness was 5 white blood cells (WBC) per μl, 14 (41.2%) of the 34 CSF samples were classified as early-stage cases (Table 2). Thus, 4 early-stage cases, which were both blood-and CSF-negative by microscopy, were PCR-positive. Furthermore, 3 (15%) of the 20 CSF samples, classified as late-stage cases since they had 6 WBC per μl in the CSF, were parasitologically positive in their corresponding blood samples but negative in the CSF by double centrifugation, and were PCR-negative, which suggests that they were in fact early cases. However, two (10%) late-stage cases, which had trypanosomes in both blood and CSF, were also positive by PCR (Table 2), indicating concordance of the results. It should be emphasized that all the 13 CSF samples, which were parasitologically positive, were also positive by PCR, demonstrating the uniformity of the results. Furthermore, 8 (38.1%) of the 21 CSF samples that were parasitologically negative were positive by PCR (Table 3), suggesting that they were late-stage sleeping sickness cases and also indicating that PCR had greater sensitivity. Also, 4 (50%) of the 8 parasitologically negative but PCR-positive CSF samples (Table 4) had <5 WBC per μl, which classified them as early cases according to the present criteria for staging sleeping sickness cases. However, 4 (28.6%) of the 14 CSF samples with ⩽5 WBC per μl were positive by PCR, suggesting that they were late-stage cases. The remaining 10 (71.4%) were negative by PCR, confirming again that they were early cases.

Statistical analysis showed good correlations between the results of HCT and double centrifugation of CSF versus the PCR results in that all samples that were parasitologically positive by these methods were also positive by PCR. The probability of unrelatedness between the white blood cell counts and the PCR methods in staging sleeping sickness patients is less than 0.005 ($P$ <0.005), indicating a

Table 1. **Comparison of the results of polymerase chain reaction (PCR), card agglutination test for trypanosomiasis (CATT) and the haematocrit centrifugation technique (HCT) in the diagnosis of trypanosomes in 35 blood samples from suspected sleeping sickness patients**

| | PCR | | Total |
|---|---|---|---|
| | No. positive | No. negative | |
| **CATT**[a] | | | |
| No. positive | 20 | 13 | 33 |
| No. negative | 0 | 2 | 2 |
| **Total** | **20** | **15** | **35** |
| **HCT**[b] | | | |
| No. positive | 14 | 0 | 14 |
| No. negative | 6 | 15 | 21 |
| **Total** | **20** | **15** | **35** |

[a] Pearson's $\chi^2$ test = 2.8283; $P$ = 0.093. Fisher's exact test = 0.176. The values are not significant even at the 5% level. There is no relationship between CATT and PCR results.

[b] Pearson's $\chi^2$ test = 17.5; $P$ <0.005. Fisher's exact test one-tailed, $P$ value = 0.0002; and two-tailed, $P$ value = 0.0005. The values are significant. There is a very strong relationship between the HCT and PCR results.

Table 2. **Results of the polymerase chain reaction (PCR) using TBR 1 and TBR 2 primers on cerebrospinal fluid (CSF) samples**

| Sample size | CSF white blood cell counts per μl | Blood | CSF | PCR | Present staging of SS case[a] | Possible stage of SS case[a] |
|---|---|---|---|---|---|---|
| 2 | | + | − | − | Early | Early |
| 4 | 0–5 | − | − | + | Early | Late |
| 8 | | − | − | − | Early | Early |
| 3 | | + | − | − | Late | Early |
| 3 | | − | − | + | Late | Late |
| 11 | >6 | − | + | + | Late | Late |
| 2 | | + | + | + | Late | Late |
| 1 | | + | − | + | Late | Late |

[a] SS = sleeping sickness.

Table 3. **Comparison of the results of double centrifugation and polymerase chain reaction (PCR) in the detection of trypanosomes in 34 cerebrospinal fluid (CSF) samples**

| Double centrifugation[a] | PCR | | Total |
|---|---|---|---|
| | No. positive | No. negative | |
| No. positive | 13 | 0 | 13 |
| No. negative | 8 | 13 | 21 |
| **Total** | **21** | **13** | **34** |

[a] Pearson's $\chi^2$ test = 13.0294; $P$<0.005. Fisher's exact test one-tailed, $P$ value = 0.00022; and two-tailed, $P$ value = 0.0006. The values are significant. There is a strong relationship between the double centrifugation and PCR results.

Table 4. **Comparison of white blood cell counts and polymerase chain reaction (PCR) in staging sleeping sickness patients**

| CSF[a] | PCR | | Total |
|---|---|---|---|
| | No. positive | No. negative | |
| Early stage (⩽5 cells per μl) | 4 | 10 | 14 |
| Late stage (6 cells per μl) | 17 | 3 | 20 |
| **Total** | **21** | **13** | **34** |

[a] Pearson's $\chi^2$ test = 11.104; $P$ <0.005. Fisher's exact test one-tailed, $P$ value = 0.00131; and two-tailed, $P$ value = 0.139. The values are significant. There is a strong relationship between the currently used criteria for staging sleeping sickness patients and the PCR results.

strong correlation between the currently used criteria for staging sleeping sickness patients and the PCR technique.

## Discussion

In the present study, two criteria for staging sleeping sickness patients were used: the presence of trypanosomes and the WBC count in cerebrospinal fluid (CSF). The protein level in CSF was not determined. The results of using these criteria were compared with PCR to determine the most suitable method. For the 35 blood samples and 34 CSF samples analysed using these methods, PCR was the most sensitive in identifying trypanosome parasites in blood and CSF. Trypanosomes were detected in 20 of 35 (57.1%) blood samples and 21 of 34 (61.8%) CSF samples by PCR; of these, 6 blood samples and 8 CSF samples were aparasitaemic.

All 14 parasitologically positive blood samples were also positive by PCR, demonstrating good concordance. However, 6 of the 21 blood samples that were negative by microscopy (HCT, MAECT and TBS) were positive by PCR, which suggests that either the characteristically low parasitaemia in *T. b. gambiense* infections could not be detected in these blood samples by microscopic techniques or that they were very early infections. The two blood samples that were negative both parasitologically and by CATT were also negative by PCR, demonstrating the specificity of PCR in identifying trypanosome parasites. However, statistical analysis ($\chi^2$ test = 2.8286; $P$ = 0.093) of the data in Table 1 showed that there was no correlation between the CATT and PCR results.

Analysis by PCR of 34 CSF samples from sleeping sickness patients showed that all the 13 parasitologically positive CSF samples were also positive by PCR, which agreed with the parasitological results. All the 13 parasitologically positive CSF samples had >5 WBC per μl and were classified as late-stage cases. Usually, 5 WBC per μl is considered to be the cut-off point in the staging and treatment of sleeping sickness patients (*12*), which is used exclusively for therapeutic reasons because the drug used to treat first-stage cases has low toxicity and does not cure the second stage of the disease. Melarsoprol, the drug used to treat second-stage sleeping sickness, is highly toxic, provoking reactive encephalopathy in 5–10% of patients with a mortality rate of 1–5%. However, use of this criterion may be associated with high probability of missing some late-stage cases with <5 WBC per μl as well as identifying false late-stage cases due to a rise in cell counts induced by infections other than sleeping sickness (*13*). With a cut-off point of 5 WBC per μl for early-stage sleeping sickness, 14 of the 34 CSF samples were classified as early cases. However, analysis of these 14 early-stage cases by PCR revealed trypanosome infection in 4 CSF samples, which suggests that, probably in some early cases with low cell counts, trypanosomes had already passed the blood–brain barrier and penetrated into the central nervous system (CNS), CSF was contaminated with blood during lumbar puncture, or the positive PCR test was due to other factors. However, based on a number of repeated experiments, the occurrence of a positive PCR test due to other factors is minimized although not completely eliminated because, when parasites are not seen in any of the body fluids, a positive PCR test is not evidence for trypanosomiasis. Nevertheless, all the 4 CSF samples with <5 WBC per μl were from suspected sleeping sickness patients who were CATT-positive, but trypanosomes could not be demonstrated in either the blood or CSF by TBS, HCT or double centrifugation. This could probably have been due to a low parasitaemia, which could not be detected by these methods. Furthermore, 17 of the 20 CSF samples classified as late-stage cases were positive by PCR, of which 13 were also parasitologically positive, showing that they were late-stage cases, while the positive PCR tests for the remaining 4 aparasitaemic CSF samples suggested CNS involvement. However, some sleeping sickness patients whose blood samples were positive by CATT and by microscopy had no trypanosomes in their CSF, while others with zero or with >6 WBC per μl were all negative by PCR, suggesting that they were early cases (Table 2). Noteworthy are the 3 sleeping sickness cases whose blood samples were positive by microscopy and who had >6 WBC per μl, while the CSF was negative by both double centrifugation and PCR, indicative of no CNS involvement despite the high number of WBC. It appears, therefore, that high or low WBC counts may not necessarily imply CNS involvement.

In an earlier study by Cattand et al. (*14*), where CSF analysis by double centrifugation and WBC counts was used to stage sleeping sickness patients, there was no correlation between the presence of trypanosomes and the number of WBC in CSF. Our study has demonstrated a strong correlation between double centrifugation and the PCR results, since parasitologically positive CSF samples were also positive by PCR. Furthermore, PCR seems to be the most sensitive diagnostic method since it detected trypanosomes in 6 blood samples and 8 CSF samples that were negative parasitologically, and it could therefore be a useful method for determining which

suspected sleeping sickness patients should be followed up by parasitological examinations for evidence of the presence of trypanosomes in their blood and/or CSF samples. The implications for treatment of sleeping sickness cases that are classified as early or late by the WBC count, but as late or early by PCR, need to be investigated in order to know whether these patients could be treated successfully with the first-stage drug alone.

In conclusion, it is imperative to carry out a detailed clinical study on the use of PCR for trypanosomiasis diagnosis and staging of patients in order to demonstrate the relation between the PCR and the outcome of treatment. ■

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## Résumé

### Mise en évidence de trypanosomes par amplification génique (PCR) chez des malades ougandais présumés atteints de trypanosomiase

Le diagnostic de la maladie du sommeil (trypanosomiase) se heurte à une difficulté qui tient aux fluctuations du degré de parasitémie. Dans la présente étude, nous avons procédé par amplification génique (PCR) pour mettre en évidence une infection trypanosomienne dans 20 échantillons de sang sur 35 (57,1%) et dans 21 échantillons de liquide céphalo-rachidien (LCR) sur 34 (61,7%) prélevés dans une région du nord-ouest de l'Ouganda où la trypanosomiase est endémique. Au total, 14 échantillons de sang et 13 échantillons de LCR qui, par double centrifugation, s'étaient révélé contenir des trypanosomes, se sont également montrés positifs par la PCR, ce qui indique une bonne concordance entre les deux méthodes. Toutefois, 6 des 21 échantillons de sang (28,6%) négatifs du point de vue parasitologique ont été donnés positifs par la PCR et 8 des 21 échantillons de LCR (38,0%), qui étaient apparus négatifs par double centrifugation, ont été donnés positifs par la PCR. On se trouve donc en présence de 14 échantillons négatifs qui pourraient provenir de cas de trypanosomiase, même si une positivité à la PCR n'est pas la preuve absolue de la présence de trypanosomes. Sur ces 8 échantillons de LCR, 4 avaient été qualifiés de cas aux premiers stades en se basant sur l'absence de trypanosomes et un nombre de leucocytes $\leqslant$ 5 par μl. Il s'ensuit que selon les critères actuels, certains cas avancés auraient pu nous échapper et il importe donc d'effectuer des essais cliniques pour voir s'il serait possible de traiter avec succès ces malades en utilisant uniquement le médicament adapté aux premiers stades de la maladie. Les 4 échantillons de LCR restants avaient été considérés comme des cas avancés, en se basant sur le fait que le nombre de leucocytes était > 6 par μl, en dépit de l'impossibilité de mettre des trypanosomes en évidence par double centrifugation. Il existe une règle empirique pour la stadification de la trypanosomiase, selon laquelle la valeur limite se situe à 5 leucocytes par μl ; il semble que cette règle conduise à laisser échapper un certain nombre de cas avancés puisque des trypanosomes ont été mis en évidence dans 4 échantillons de LCR provenant de cas présumés ayant un nombre de leucocytes inférieur à 5 par μl.

A noter les 3 cas de trypanosomiase dont les échantillons de sang étaient positifs à l'examen microscopique et dont le LCR contenait > 5 leucocytes par μl, mais qui se sont montrés négatifs par double centrifugation et amplification génique. Ces résultats sont révélateurs d'une absence d'atteinte du système nerveux central malgré un nombre élevé de leucocytes. On constate donc qu'un nombre élevé de leucocytes ne signe pas forcément une atteinte du système nerveux central.

Lors d'une analyse précédente au cours de laquelle nous avons soumis les échantillons de LCR à une double centrifugation en procédant également à une numération leucocytaire pour déterminer le stade de la maladie, nous n'avons constaté aucune corrélation entre la présence de trypanosomes et le nombre de leucocytes dans le LCR. Notre étude montre qu'il existe en revanche une forte corrélation entre les résultats de la double centrifugation et ceux de l'amplification génique puisque les échantillons de LCR positifs à l'examen parasitologique l'étaient également par la PCR. En outre, il semble que la PCR soit la méthode de diagnostic la plus sensible car elle a permis de déceler des trypanosomes dans 6 échantillons de sang et 8 échantillons de LCR négatifs à l'examen parasitologique. Elle pourrait donc être utile pour déterminer quels sont les malades présumés atteints de trypanosomiase qu'il faut mettre en observation. Il faudrait également voir ce que ces résultats impliquent sur le plan thérapeutique pour des trypanosomiases jugées aux premiers stades d'après la double centrifugation mais qui seraient à un stade avancé selon la PCR.

## Resumen

### Detección de tripanosomas mediante la reacción en cadena de la polimerasa en presuntos afectados por la enfermedad del sueño en Uganda

El diagnóstico de la enfermedad del sueño (tripanosomiasis) resulta difícil debido a la fluctuación de la parasitemia que muestran los afectados por esa enfermedad. En el presente estudio, los análisis realizados mediante la reacción en cadena de la polimerasa (RCP) demostraron la existencia de infección tripanosómica en 20 de 35 (57,1%) muestras sanguíneas y 21 de 34 (61,7%) muestras de líquido cefalorraquídeo (LCR) obtenidas en una zona de endemicidad de la enfermedad en el noroeste de Uganda. En total, 14 muestras de sangre y 13 de LCR que habían dado positivo en la doble centrifugación dieron también positivo en la prueba de RCP, lo que reveló una buena concordancia entre los dos métodos. Sin embargo, 6 (28,6%) de las 21 muestras sanguíneas negativas en el análisis parasitológico dieron positivo en la prueba de RCP, y 8 (38,0%) de las 21 muestras de LCR negativas en la doble centrifugación dieron positivo en la RCP. Cabe deducir que esas 14 muestras negativas podían corresponder a casos de enfermedad del sueño, aunque una RCP positiva no prueba la existencia de tripanosomas. De las 8 muestras de LCR, 4 correspondían a casos calificados de iniciales en vista de la ausencia de tripanosomas y del recuento leucocitario $\leqslant$ 5 por μl. Esto lleva a pensar que algunos de los pacientes que se hallan en la fase avanzada podrían pasar desapercibidos con los criterios actuales, de ahí la necesidad de llevar a cabo ensayos clínicos para determinar si es posible tratar eficazmente esos casos empleando sólo la medicación establecida para la fase inicial. Las 4 muestras restantes de LCR correspondían a casos clasificados como avanzados dada la existencia de un recuento leucocitario superior a 6 por μl, aun cuando la doble centrifugación no reveló tripanosomas en esas muestras. Al parecer el valor discriminante de 5 leucocitos por μl usado como regla empírica para determinar la fase de la enfermedad del sueño no permite detectar algunos casos avanzados, pues se detectaron tripanosomas en 4 muestras de LCR de casos presuntos con menos de 5 leucocitos por μl.

Son de destacar los 3 casos que, con muestras sanguíneas positivas en la microscopía y más de 5 leucocitos por μl en el LCR, eran sin embargo negativos según los resultados de la doble centrifugación y de la RCP, dato indicativo de que no habría afección del sistema nervioso central (SNC) pese al elevado número de leucocitos. Parece, por consiguiente, que un recuento leucocitario alto no entraña necesariamente afección del SNC.

En un análisis del LCR realizado anteriormente mediante doble centrifugación y recuento leucocitario para determinar la fase de la enfermedad no se observó ninguna correlación entre la presencia de tripanosomas y el número de leucocitos en el LCR. Nuestro estudio ha puesto de manifiesto una alta correlación entre los resultados de la doble centrifugación y los de la RCP, pues las muestras de LCR positivas en el análisis parasitológico dieron también positivo en la prueba de la RCP. Además, este último parece ser el método diagnóstico más sensible, pues detectó tripanosomas en 6 muestras de sangre y 8 muestras de LCR parasitológicamente negativas, y podría emplearse por tanto para determinar cuáles de los pacientes afectados por la enfermedad del sueño deben ser objeto de seguimiento. Es necesario investigar qué repercusiones tiene eso para el tratamiento de los casos de enfermedad del sueño que pueden diagnosticarse como iniciales según la doble centrifugación y como avanzados según la RCP.


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