

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



Balasegaram, M; Harris, S; Checchi, F; Hamel, C; Karunakara, U (2006) Treatment outcomes and risk factors for relapse in patients with early-stage human African trypanosomiasis (HAT) in the Republic of the Congo. *Bulletin of the World Health Organization*, 84 (10). pp. 777-82. ISSN 0042-9686 DOI: <https://doi.org/10.2471/BLT.05.028399>

Downloaded from: <http://researchonline.lshtm.ac.uk/10607/>

DOI: [10.2471/BLT.05.028399](https://doi.org/10.2471/BLT.05.028399)

Usage Guidelines

Please refer to usage guidelines at <http://researchonline.lshtm.ac.uk/policies.html> or alternatively contact researchonline@lshtm.ac.uk.

Available under license: <http://creativecommons.org/licenses/by/2.5/>

Research

Treatment outcomes and risk factors for relapse in patients with early-stage human African trypanosomiasis (HAT) in the Republic of the Congo

Manica Balasegaram,^a Steve Harris,^a Francesco Checchi,^b Catherine Hamel,^a & Unni Karunakara^a

Objective In 2002–03, the Republic of the Congo increased the threshold separating stage 1 and 2 cases of human African trypanosomiasis (HAT) from a cerebrospinal fluid (CSF) white cell count of 5 cells/mm³ to 10 cells/mm³. We aimed to assess whether the increased threshold of 10 cells/mm³ is a safe indicator of stage 2 disease.

Methods We assessed patients treated for stage 1 HAT caused by *Trypanosoma brucei gambiense* in the Republic of the Congo between April 2001 and April 2005. Patients with 0–10 cells/mm³ in CSF were classed as stage 1 and treated with pentamidine. Patients with CSF of > 10 cells/mm³ were classed as stage 2 and treated with either melarsoprol or eflornithine. We did a retrospective analysis of all patients treated after the September 2002 increase in threshold for classification of HAT disease stage 2, and who were eligible for at least 1 year of follow-up. Primary outcome was survival without death or relapse within 1 year of discharge. Risk factors for treatment failure, in particular CSF white cell count on diagnosis, were assessed.

Findings Between September 2002 to April 2004, 692 patients eligible for our analysis were treated with pentamidine. All were discharged alive. Relapse rate was 5% ($n=33$). The only identified risk factor for relapse was a CSF white cell count of 6–10 cells/mm³ rather than 0–5 cells/mm³ (adjusted hazard ratio 3.27 (95% confidence interval, 1.52–7.01); $P=0.002$).

Conclusion A threshold of 5 white cells/mm³ in CSF is safer than 10 cells/mm³ to determine stage 2 HAT and reduce risk of relapse.

Bulletin of the World Health Organization 2006;84:777–782.

Voir page 781 le résumé en français. En la página 781 figura un resumen en español.

يمكن الاطلاع على الملخص بالعربية في صفحة 781.

Introduction

Human African trypanosomiasis (sleeping sickness) caused by the parasite *Trypanosoma brucei gambiense* is endemic in the Republic of the Congo. The disease is transmitted by the tsetse fly (*Glossina* genus) and manifests itself in two stages. The early stage (stage 1) occurs shortly after initial infection and can present as a haemolymphatic illness with fever, headaches, lymphadenopathy, and organomegaly. The late stage (stage 2) presents several months or even years after infection and is characterized by central nervous system involvement. The disease is fatal if untreated.

The early and late stages of sleeping sickness are differentiated by visualization of the parasite in cerebrospinal fluid (CSF) or the proxy indicator of white cell count in CSF. Treatment varies according to stage. Pentamidine is currently the

standard recommended treatment for the early stage of the disease. This drug is a water-soluble compound given at a dose of 4 mg/kg once daily for 7 days either intramuscularly or by slow intravenous injection. Maximum plasma concentrations are attained within 1 hour of an intramuscular injection, but the drug is largely bound to plasma proteins and has a long-lasting action with a slow rate of excretion.¹ Importantly, only very low concentrations of the drug are achieved in CSF, making it unsuitable for use in confirmed stage 2 cases.²

Médecins Sans Frontières (MSF) has run three trypanosomiasis intervention programmes in conjunction with the Republic of the Congo National Programme (the Programme National de lutte contre la Trypanosomiase Humaine Africaine (PNLTHA)) in Gamboma (from April 2001), Nkayi (September 2002), and Mossaka (July 2003) in the

Plateaux, Bouenza, and Cuvette regions, respectively. The programmes ended in April 2005.

In September 2002, the MSF programmes, in accordance with a recommendation by the PNLTHA, increased the threshold separating stage 1 and 2 cases from 5 cells/mm³ to 10 cells/mm³. This change was accompanied by a formal revision to the PNLTHA protocol in 2003. We did a retrospective analysis in a cohort of stage 1 patients treated for human African trypanosomiasis caused by *T.b. gambiense* in the Republic of the Congo. We aimed to assess whether the use of a higher CSF white cell count threshold increased the risk of relapse.

Methods

Patients were originally enrolled through passive case detection at fixed sites, as well as by active case detection by mobile

^a Médecins Sans Frontières, 67–74 Saffron Hill, London EC1N 8QX, England. Correspondence to Dr Balasegaram (manica.balasegaram@london.msf.org).

^b Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, England.

Ref. No. 05-028399

(Submitted: 18 November 2005 – Final revised version received: 13 March 2006 – Accepted: 24 March 2006)

teams systematically screening villages. The national protocol of the PNLTHA guided diagnosis and treatment.

Diagnosis (Fig. 1) involved screening (card agglutination trypanosomiasis test (CATT) with whole blood), confirmation (visualization of the parasite in blood or lymph, or a positive result with CATT dilution of 1 in 8), and staging (parasite found in CSF or CSF white cell count).

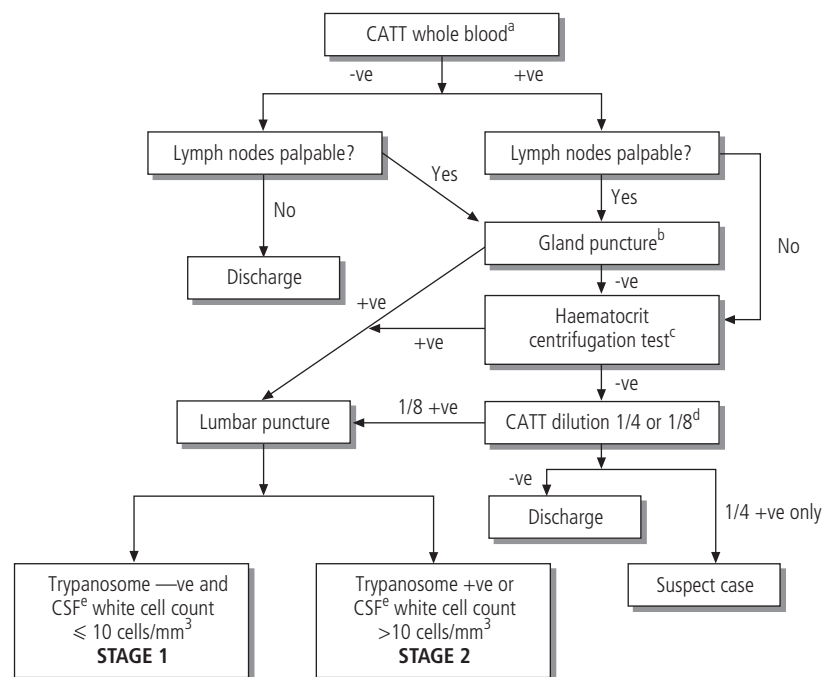
We analysed risk factors for treatment failure during the first year of follow-up using Cox proportional hazards regression. Patients were included in the analysis if they had a stage 1 diagnosis, were treated with pentamidine (4mg/kg by intramuscular (IM) injection once daily for 7 days), admitted on or after September 2002 (the month when the stage 1 to stage 2 white cell count threshold was raised), and eligible for 1 year of follow-up. Patients were excluded if they had not attended at least one follow-up visit or if baseline variables such as age, sex, parasitological findings and treatment outcome were not in their records.

We restricted our analysis to the first year of follow-up, since follow-up rates were unacceptably low for longer periods. We took any visit from month 10 (day 304) to month 14 (day 425) after discharge as the 1-year follow-up visit. We defined failure as death attributable to any cause after discharge, recurrence of parasites in any body fluid, CSF white cell count > 50 cells/mm³ and at least doubled from the previous measurement, or white cell count 11–49 cells/mm³ with either a significant increase from the previous measurement or with symptoms suggestive of sleeping sickness.^{3–5}

Potential risk factors (treatment centre, sex, age, screening mode, technique on which parasitological confirmation was based (i.e. visualization of parasite or serological alone), CSF white cell count) and confounders (period during which the patient was admitted) were entered in the multivariate model if associated with the outcome at the $P < 0.20$ level in a univariate analysis. We forced semester of admission into the final model since pentamidine relapse rates varied over the duration of the project (data not shown). We also tested for interactions between covariates and verified the proportionality assumption.

Data were entered at programme locations into either an Excel database (Gamboma) or YoTryps (Nkayi and Mossaka), a Microsoft Access-based

Fig. 1. Scheme showing the various steps used in diagnosis of study cases



^a CATT = Card agglutination trypanosomiasis test (whole blood).

^b Gland puncture = lymph node (aspiration).

^c Haematocrit centrifugation test is also known as the Woo test.

^d Serological cases are those that are CATT 1/8 positive but have no trypanosomes detected in cerebrospinal fluid.

^e CSF = cerebrospinal fluid.

software designed for human African trypanosomiasis programmes. Data were analysed with Stata version 8.0 (Stata Corp, College Station, Texas, USA).

Ethical approval

The study was a retrospective analysis on Médecins Sans Frontières' operational medical work in the Republic of the Congo. Approval for data exportation, analysis and reporting was obtained from the PNLTHA of the Republic of the Congo. The data sets extracted and used for analysis were anonymized through removal of all patients' names, separation into a new dataset, and analysis by a statistician unconnected with the programmes.

Results

Between April 2001 and March 2005, 1986 patients were diagnosed with primary stage 1 human African trypanosomiasis. Criteria for selection of the cohort retained for analysis is shown in Fig. 2. Of 692 patients discharged alive after treatment with pentamidine, 454 (66%) and 371 (54%) attended follow-up at 6 months (± 2 months) and one year (± 2 months), respectively. Overall, 652 patients (94%) attended at least one

follow-up visit and were included in the multivariate analysis. Table 1 shows their baseline characteristics.

Patients not attending at 1-year follow-up differed significantly from those who did attend in the semester of admission: 159 of 228 patients (70%) admitted in September 2002 to March 2003 were seen, compared with 212 of 424 (50%) later; $P < 0.001$. Groups did not differ with respect to any other baseline characteristics.

There were 33 treatment failures (5% of 652 eligible for analysis). Age, sex, mode of screening and technique of diagnosis (parasitological or serological) did not affect risk of failure (Table 1). The proportion of failures was higher in patients with a white cell count of 6–10 cells/mm³ than in those with white cell count of 0–5 cells/mm³ (10 of 84 (12%) versus 23 of 568 (4%) eligible for analysis). Patients with a white cell count of 6–10 cells/mm³ had a similar median duration of follow-up to those with white cell count of 0–5 cells/mm³ (316 versus 338 days; $P = 0.880$). Among the 41 defaulters, 18 diagnoses (44%) were serological in the 6–10 cells/mm³ group compared with 165 of 240 (69%) in the 0–5 cells/mm³ group ($P = 0.004$);

defaulters in the two exposure groups were otherwise similar.

Patients treated with pentamidine with a raised white cell count (6–10 cells/mm³) had a significantly higher risk of failure (adjusted hazard ratio 3.27; $P=0.002$) than did those with a white cell count of 0–5 cells/mm³ (Table 1). The association remained significant when stratified by treatment centre: 2.75 (95% confidence interval (CI), 0.99–7.67) in Nkayi and 4.48 (95% CI: 1.29–15.52) in Mossaka.

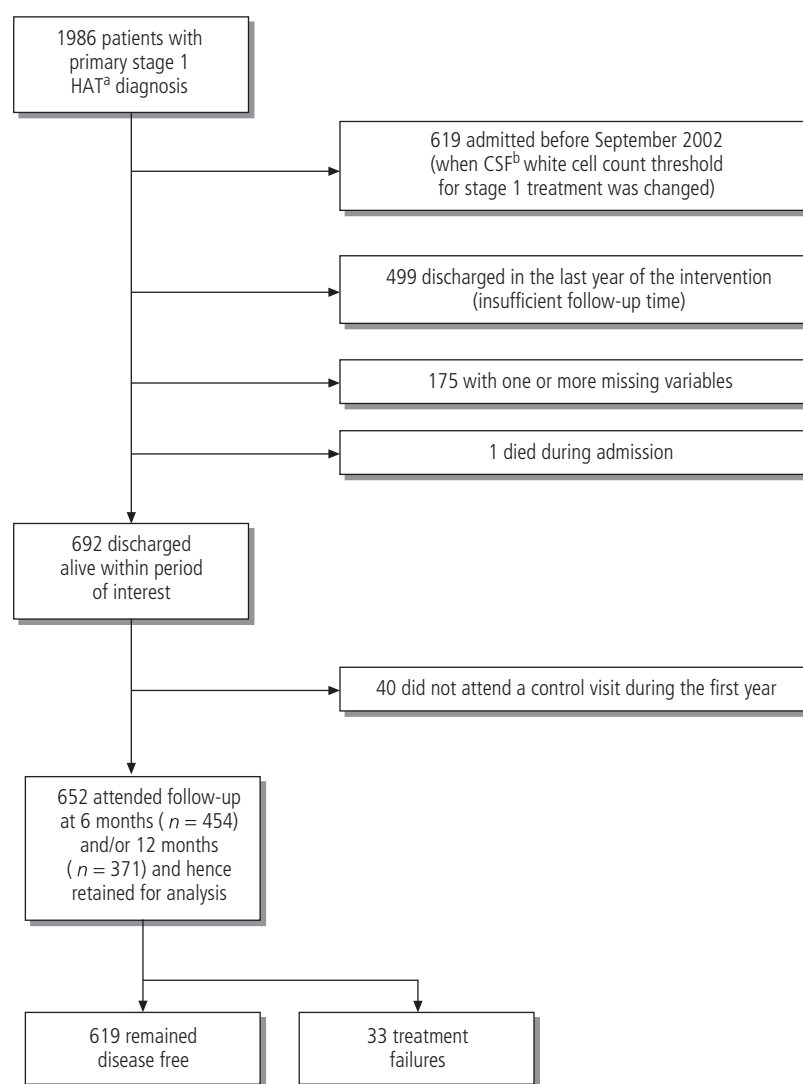
Discussion

Our results show that the risk of treatment failure in patients with a CSF white cell count of 6–10 cells/mm³ is three times higher than in those with a count of 0–5 cells/mm³. This difference may be because some of these patients had late stage or stage 2 disease for which pentamidine treatment is ineffective.

Attempts have been made to use pentamidine to treat patients with a mild elevation of CSF white cell count.⁶ Doua et al.⁷ in Côte d'Ivoire, for example, showed that pentamidine could be effective in "intermediate" cases with a CSF white cell count between 6 cells/mm³ and 20 cells/mm³. However, Lejon et al.⁸ in Uganda noted a higher risk of relapse for these patients treated with pentamidine⁸ and went on to recommend a value of 10 cells/mm³ as a more appropriate cut off. Ruiz et al.⁹ indeed found that a CSF white cell count of 6–10 cells/mm³ was not associated with a higher risk of relapse in patients treated with pentamidine.⁹ Our results somewhat contradict outcomes from these studies and indicate that a lower threshold of 5 cells/mm³ may be advisable in the Republic of the Congo.

A CSF white cell count cut-off of 10 cells/mm³ to differentiate stage 1 and 2 cases is a feature of the national diagnostic protocol of the PNLTHA. Many protocols use a cut-off point of 5 cells/mm³. The reason for adopting a high threshold is because melarsoprol, which is highly toxic, was initially used for the treatment of stage 2 cases. This higher threshold seems justified in view of the fact that if the lower threshold of 5 cells/mm³ had been used, an additional 84 patients (in the analysed group) would have been treated with potentially toxic stage 2 medication. Since about 5% of all patients treated with melarsoprol die from severe adverse events, we calculate that treatment of these 84

Fig. 2. Criteria used for selecting the cohort retained for analysis



^a HAT = human African trypanosomiasis.

^b CSF = cerebrospinal fluid.

extra patients with melarsoprol would have resulted in 4 additional deaths to prevent 10 relapses.

However, we feel that where an alternative to melarsoprol, such as eflornithine is available, use of a higher threshold is not justified.¹⁰ In our programme in the Republic of the Congo we also found a relatively low case fatality rate of 1.7% ($n=5$) among 288 patients treated with eflornithine. Furthermore, the idea that one can have a positive risk–benefit with a higher CSF white cell count is based on the assumption that all patients can be followed up and any extra relapses can be retreated. In our programme, despite additional resources not normally available to the PNLTHA, only 54% of patients attended follow-up at 1 year. In areas where good follow-up rates can

not be guaranteed, additional deaths in the community may occur in patients who relapse or who are lost or unable to attend follow-up.

Overall, the treatment failure rate (5%) in our programme was acceptable and similar to levels noted in other studies.^{6,11,12} A high proportion (62%) of patients entered in the analysis of relapse risk were diagnosed serologically, in the absence of visualized parasites. This low rate of parasite detection may be related to poor laboratory performance. As such, the treatment of serological cases with a CAT end titre > 1/8 has been proposed as a control strategy in areas with a high prevalence of sleeping sickness.¹³ However, many serological cases may not be true cases at all and positive results may be the result of, for example, presumed

Table 1. Baseline characteristics of patients and risk factors for treatment failure within first year after discharge in stage 1 patients treated with pentamidine

Baseline characteristic/ potential risk factor	Patients (n=652)	Relapses	Crude hazard ratio (95%CI) ^b	Adjusted hazard ratio ^a (95%CI)
Treatment centre				
Nkayi	457 (70%)	23	1.00	–
Mossaka	195 (30%)	10	1.15 (0.54–2.42)	–
Semester of admission				
September 2002 to March 2003	228 (35%)	13	1.00	1.00
April to September 2003	217 (33%)	13	1.04 (0.48–2.27)	0.86 (0.39–1.91)
October 2003 to March 2004	207 (32%)	7	0.66 (0.26–1.65)	0.55 (0.22–1.40)
Sex				
Male	351 (54%)	19	1.00	–
Female	301 (46%)	14	0.90 (0.45–1.77)	–
Age				
≥ 15 years	504 (77%)	23	1.00	–
< 15 years	148 (23%)	10	1.47 (0.70–3.09)	–
Mode of screening				
Active	490 (75%)	26	1.00	–
Passive	162 (25%)	7	0.69 (0.30–1.60)	–
Technique on which diagnosis and staging was based				
Direct gland puncture	157 (24%)	9	1.00	–
Centrifugation (Woo or QBC ^c)	98 (15%)	7	1.11 (0.41–3.00)	–
CATT positive at dilution 1/8 or higher	396 (61%)	17	0.83 (0.37–1.87)	–
White cell count in CSF^d				
0–5 cells/ mm ³	568 (87%)	23	1.00	1.00
6–10 cells/ mm ³	84 (13%)	10	3.05 (1.45–6.40)	3.27 (1.52–7.01)

^a Adjusted hazard ratios based on Cox regression model with $P=0.030$ (goodness of fit).

^b CI = confidence interval.

^c QBC = quantitative buffy coat.

^d CSF = cerebrospinal fluid.

cases having been treated several years ago. There is evidence of immunity following *T.b. gambiense* infection and it is also known that results of the CATT may remain positive for some time.^{14,15} Furthermore, the positive predictive value of the CATT dilutions is unknown and may vary over the length of a control programme. Perhaps a higher threshold of even 1/16 or 1/32 should be considered especially when the overall prevalence of the disease is moderate to low.

Our results should be interpreted with some caution since they are derived from a retrospective analysis, and are not from a randomized study but from a field-based medical intervention. Additionally, our data were insufficient

to analyse outcomes beyond 1 year of follow-up; patients can relapse up to 3 years after initial treatment.¹¹

Conclusion

Overall, pentamidine was safe and well tolerated. However, a CSF white cell count of 10 cells/mm³ as the threshold between stage 1 and 2 cases seems to be associated with a higher risk of relapse. Therefore, we recommended that stage 1 should be defined by a CSF white cell count of 0–5 cells/mm³ (with absence of trypanosomes in the CSF) and that a count of 6 cells/mm³ and greater should be treated as a stage 2 case, especially if the alternative stage 2 drug eflornithine is available and can be safely adminis-

tered. In view of a higher risk of relapse, a higher threshold should be used in the context where good follow-up is possible. Surveillance of treated cases is important to monitor development of possible resistance to pentamidine. ■

Acknowledgements

We thank the field teams of Médecins Sans Frontières and Dr Claude Rudy Manthelot, director of the PNLTHA, for their work and assistance in the Republic of the Congo.

Competing interests: none declared.

Résumé

Résultats du traitement et facteurs de risque de rechute chez les personnes atteintes de trypanosomiase africaine humaine (TAH) à un stade peu avancé en République du Congo

Objectif Dans les années 2002-2003, les responsables sanitaires de la République du Congo ont fait passer de 5 leucocytes/mm³ à 10 leucocytes/mm³ le seuil de séparation des stades 1 et 2 de la trypanosomiase africaine humaine (TAH) d'après la numération leucocytaire dans le liquide céphalorachidien. On s'est efforcé d'évaluer la sécurité du tri effectué à partir du seuil augmenté de 10 leucocytes/mm³ en tant qu'indicateur du stade 2 de la maladie.

Méthodes On a évalué des patients traités pour une TAH au stade 1 due à *Trypanosoma brucei gambiense* en République du Congo entre avril en 2001 et avril 2005. Les patients dont la numération leucocytaire dans le liquide céphalorachidien se situait entre 0 et 10 leucocytes/mm³ ont été classés «stade 1» et traités par la pentamidine. Ceux dont la numération était > 10 leucocytes/mm³ ont été classés «stade 2» et traités par du mélarsoprol ou de l'éflornithine. Une étude rétrospective a été réalisée sur l'ensemble des patients traités après l'augmentation du seuil de classification de la TAH en stade 2 effectuée en septembre 2002 et répondant aux critères d'inclusion dans un suivi d'un an au

moins. Les deux principaux résultats du traitement pris en compte étaient la survie sans décès ou la rechute dans l'année suivant la sortie de l'établissement de soins. Les facteurs de risque d'échec thérapeutique, notamment la numération leucocytaire dans le liquide céphalorachidien, ont été évalués.

Résultats Entre septembre 2002 et avril 2004, 692 patients remplissant les critères d'inclusion dans l'analyse ont été traités par la pentamidine. Tous ont quitté l'établissement de soins vivants. Le taux de rechute était de 5 % (n = 33). Le seul facteur de risque de rechute identifié était une numération leucocytaire située entre 6 et 10 leucocytes/mm³, par comparaison avec une valeur de 10 leucocytes/mm³ (rapport de risque ajusté 3,27; intervalle de confiance à 95 % : 1,52 - 7,01; p = 0,002).

Conclusion Le seuil de 5 leucocytes/mm³ dans le liquide céphalorachidien est plus sûr que celui de 10 leucocytes/mm³ pour définir une TAH au stade 2 et réduire le risque de rechute.

Resumen

Resultados terapéuticos y factores de riesgo de recaída en pacientes con tripanosomiasis africana humana (TAH) en fase inicial en la República del Congo

Objetivo En 2002-2003, la República del Congo aumentó el umbral de separación de los casos de fase 1 y 2 de tripanosomiasis africana humana (TAH) de un recuento de leucocitos en líquido cefalorraquídeo (LCR) de 5 células/mm³ a 10 células/mm³. Decidimos determinar si ese mayor umbral, de 10 células/mm³, es un indicador seguro de la fase 2 de la enfermedad.

Métodos Evaluamos a pacientes tratados contra la TAH por *Trypanosoma brucei gambiense* en fase 1 en la República del Congo entre abril de 2001 y abril de 2005. Los pacientes con 0-10 células/mm³ en LCR se clasificaron como fase 1 y fueron tratados con pentamidina. Los pacientes con más de 10 células/mm³ en LCR se clasificaron como fase 2 y fueron tratados con melarsoprol o eflornitina. Hicimos un análisis retrospectivo de todos los pacientes tratados después de aumentar el umbral de definición de la fase 2, en septiembre de 2002, y considerados aptos para un seguimiento

de al menos un año. Como variable principal de valoración se empleó la supervivencia sin defunción o recaída durante el primer año tras el alta. Se evaluaron los factores de riesgo de fracaso terapéutico, en particular el recuento de leucocitos en LCR en el momento del diagnóstico.

Resultados Entre septiembre de 2002 y abril de 2004, 692 pacientes aptos para nuestro análisis fueron tratados con pentamidina. Todos sobrevivieron y recibieron el alta. La tasa de recaídas fue del 5% (n=33). El único factor de riesgo conocido fue un recuento de leucocitos en LCR de 6-10 células/mm³ en lugar de 0-5 células/mm³ (razón de riesgos instantáneos ajustada = 3,27 (intervalo de confianza del 95% = 1,52-7,01); P=0,002).

Conclusiones La cifra de 5 leucocitos/mm³ en el LCR es más segura que 10 leucocitos/mm³ como umbral para determinar la fase 2 de la TAH y reducir el riesgo de recaída.

ملخص

حصائل المعالجة وعوامل خطر النكس لدى مرضى المرحلة الباكرة من داء المثقبيات البشري الأفريقي في جمهورية الكونغو

الخلايا في السائل النخاعي لديهم عن 10 خلايا لكل مليمتري مكعب في المرحلة الثانية وعولجوا بالميلارسبرول أو بالإيفلورنيثين. وأجرينا تحليلاً استعادياً لكل المرضى الذين عولجوا في الفترة التي تلت زيادة عتبة تصنيف المرضى بداء المثقبيات البشري الأفريقي ضمن المرحلة الثانية، بعد شهر أيلول/سبتمبر 2002، وممن كانوا مؤهلين للمتابعة لمدة سنة. وقد كانت الحصيلة الأولية هي البقاء على قيد الحياة وعدم حدوث نكس بعد مرور سنة على التخرج من المستشفى، كما قِيمنا عوامل الخطر لفشل المعالجة، ولاسيما أثر عدد الخلايا (الكريات) البيضاء في السائل النخاعي على التشخيص.

الموجودات: عولج 692 مريضاً مؤهلاً للدراسة التحليلية للمعالجة بالبنتاميدين في الفترة بين أيلول/سبتمبر 2002 ونيسان/إبريل 2004. وقد

الهدف: في عامي 2002-2003 زادت جمهورية الكونغو من عتبة الفصل بين المرحلة الأولى والمرحلة الثانية في تشخيص حالات داء المثقبيات البشري الأفريقي لتصبح 10 خلايا لكل مليمتري مكعب من السائل النخاعي بعد أن كانت 5 خلايا لكل مليمتري مكعب. وهدفنا تقييم فيما إذا كانت هذه الزيادة من المؤشرات المأمونة للمرحلة الثانية من هذا الداء.

الطريقة: أجرينا تقييماً لمرضى عولجوا لإصابتهم بالمرحلة الأولى من داء المثقبيات البشري الأفريقي الناجم عن المثقبيات البروسية الغامبية في جمهورية الكونغو في الفترة بين نيسان/إبريل 2001 ونيسان/إبريل 2005. وقد صُنّف المرضى الذين كان لديهم 0-10 خلايا لكل مليمتري مكعب من السائل النخاعي ضمن المرحلة الأولى وعولجوا بالبنتاميدين، فيما صُنّف المرضى الذين زاد عدد

الاستنتاج: إن عتبة 5 خلايا لكل مليمتري مكعب من السائل النخاعي أكثر أماناً من 10 خلايا لكل مليمتري مكعب من السائل النخاعي في تشخيص المرحلة الثانية من داء المثقبيات البشري الأفريقي، وهذه العتبة ستقصر من خطر النكس.

تخرج جميعهم من المستشفى أحياناً، وكان معدل النكس 5% (33 مريضاً) وكان عامل الخطر الوحيد للنكس هو عدد الكريات البيض الذي يتراوح بين 6-10 خلايا لكل مليمتري مكعب بدلاً من 5-0 خلايا/مليمتري مكعب. إذ بلغ معدل الخطر المُصَحَّح 3.27 (بفاصلة ثقة 95% لقيم تتراوح بين 1.52 و7.01، وبقيمة احتمال 0.002).

References

- Sands M, Kron MA, Brown RB. Pentamidine: a review. *Rev Infect Dis* 1985;7:625-34.
- Bronner U, Doua F, Ericsson O, Gustafsson LL, Miezán TW, Rais M. Pentamidine concentrations in plasma, whole blood and cerebrospinal fluid during treatment of *Trypanosoma gambiense* infection in Côte d'Ivoire. *Trans R Soc Trop Med Hyg* 1991;85:608-11.
- Burri C, Nkunku S, Merolle A, Smith T, Blum J, Brun R. Efficacy of new, concise schedule for melarsoprol in treatment of sleeping sickness caused by *Trypanosoma brucei gambiense*: a randomised trial. *Lancet* 2000; 356:1419-25.
- Pepin J, Khonde N, Maiso F, Doua F, Jaffar S, Ngampo S. Short-course eflornithine in Gambian trypanosomiasis: a multi-centre randomized controlled trial. *Bull World Health Organ* 2000;78:1284-95.
- Lejon V, Buscher P. Stage determination and follow-up in sleeping sickness. *Med Trop (Mars)* 2001;61:355-60.
- Pepin J, Milord F. The treatment of human African trypanosomiasis. *Adv Parasitol* 1994;33:1-47.
- Doua F, Miezán TW, Sanon Singaro JR, Boa Yapo F, Baltz T. The efficacy of pentamidine in the treatment of early-late stage *Trypanosoma brucei gambiense* trypanosomiasis. *Am J Trop Med Hyg* 1996;55:586-8.
- Lejon V, Legros D, Savignoni A, Etchegorry MG, Mbulamberi D, Buscher P. Neuro-inflammatory risk factors for treatment failure in "early second stage" sleeping sickness patients treated with pentamidine. *J Neuroimmunol* 2003;144:132-8.
- Ruiz JA, Simarro PP, Josenando T. Control of human African trypanosomiasis in the Quicama focus, Angola. *Bull World Health Organ* 2002;80:738-45.
- Chappuis F, Udayraj N, Stietenroth K, Meussen A, Bovier PA. Eflornithine is safer than melarsoprol for the treatment of second-stage *Trypanosoma brucei gambiense* human African trypanosomiasis. *Clin Infect Dis* 2005; 41:748-51.
- Control and surveillance of African Trypanosomiasis: report of a WHO expert committee. Geneva: World Health Organization; 1998.
- Pepin J, Khonde N. Relapses following treatment of early stage *Trypanosoma brucei gambiense* sleeping sickness with a combination of pentamidine and suramin. *Trans R Soc Trop Med Hyg* 1996;90:183-6.
- Simarro PP, Ruiz JA, Franco JR, Josenando T. Attitude towards CATT-positive individuals without parasitological confirmation in the African Trypanosomiasis (*T.b.gambiense*) focus of Quicama (Angola). *Trop Med Int Health* 1999;4:858-61.
- Khonde N, Pepin J, Niyonsenga T, Milord F, De Wals P. Epidemiological evidence for immunity following *Trypanosoma brucei gambiense* sleeping sickness. *Trans R Soc Trop Med Hyg* 1995;89:607-11.
- García A, Jamonneau V, Magnus E, Laveissiere C, Lejon V, N'Guessan P, et al. Follow-up of Card Agglutination Trypanosomiasis Test (CATT) positive but apparently aparasitaemic individuals in Côte d'Ivoire: evidence for a complex and heterogeneous population. *Trop Med Int Health* 2000;5:786-93.