SM Online
Scientific Resources

SM Journal of Infectious Diseases

Research Article

Relapse Versus Non Relapse in Human African Trypanosomiasis: Simple Criteria for Discrimination

Itakala FB[1], Kayembe JMNT[2], Mananga GI[3] and Mashinda DK[4]

[1]*Department of Neurology, Faculty of Medicine, University of Kinshasa, Congo*
[2]*Department of Infectious diseases, Faculty of Medicine, University of Kinshasa, Congo*
[3]*Service of Infectious diseases, Department of Neurology, Faculty of Medicine, University of Kinshasa, Congo*
[4]*School of Public Heath, Faculty of Medicine, University of Kinshasa, Congo*

Article Information

Received date: Aug 25, 2016
Accepted date: Oct 20, 2016
Published date: Oct 28, 2016

***Corresponding author**

Itakala FB, Neuro-Psycho-Pathology Center, Department of Neurology, University of Kinshasa, Congo, Tel: +243 813193073; Email: felicienitakala@gmail.com

Keywords Post-Treatment Period; Stage-2 HAT; Sterile CSF; Simple Clinical and Biological Criteria; Relapse; Non-Relapse

Abstract

Background and Objective: In post-treatment phase of the Human African Trypanosomiasis (HAT), some clinical manifestations might imply a relapse resulting sometimes in unnecessary patient exposure to adverse effects of trypanocidal treatments. The aim of this study is to identify clinical and biological markers easily accessible for discrimination between relapse and non-relapse.

Methods: Retrospective analysis compared clinical and laboratory data of 20 subjects with suspected relapse and 53 non-relapses. For all of the participants the research of the trypanosome on CSF was negative by direct parasitological techniques, but they presented with abnormal clinical manifestations in post-treatment phase of HAT in stage-2. As appropriate, the following analyzes were applied with significance level of 5%: Fischer's exact test, chi-square test of Pearson and U-test of Mann-Whitney.

Results: Relapse is more likely in patients with clinical signs of progressive installation after a lag of at least three months, associated with a Cerebrospinal Fluid (CSF) profile of meningitis type, and a leukocyte count of ≥120 cells/mm3 and a score >0. Non relapse seems more feasible when symptoms appeared earlier, regardless of clinical latency, the cytology and chemistry profile of the CSF showed increased albumin level in CSF with a normal leukocyte count of ≤5 cells/mm3 and a variable score. Different phenotypes are suggested in the latter group, whose validation is essential.

Conclusion: The present investigation strongly suggests that the analysis of clinical data and the cyto-biological profile of the CSF have a discriminatory power to differentiate between relapse and non-relapse when HAT parasitological tests are negative. Multicenter investigations should be considered to strengthen the power of the observed associations.

Introduction

Relapse is classically defined as a new disease evolution, succeeding a first affection of the same nature, without a new infection. In other words, it is an endogenous infection. It was reported in the HAT in 1937 [1]. It occurs in HAT due to Trypanosoma brucei (T.b) gambiense when some parasites incubated in an organ serving as a barrier, escaped the action of an effective trypanocide and subsequently invade the central circulation [2].

The pathogenic mechanisms of this phenomenon are still subject of controversy [2,3]. However, many authors consider the brain as the main source of relapse in T.b-infection [2,3,4]. Indeed, a dense confinement of trypanosomes in the choroid plexus more than anywhere else was found in the body of experimental host (rats, mice) [2,3]. However, WOLBURG et al [5] have demonstrated experimentally in laboratory animals that the choroid plexus is a hostile environment for the survival of trypanosomes and will not allow direct colonization of the brain parenchyma. Also, the new pathogenic mechanism proposed to explain endogenous re infestation in HAT rather involves the intervention of trypanosomes located near or inside glial structures from which they can repopulate the blood vessels and disrupt the sleep-wake cycle [5].

In practice, the certainty of relapse diagnosis in the HAT is based on the identification of trypanosomes in the CSF. However, the low sensitivity of parasitological techniques makes rather this detection very scarce and difficult. This fact justifies the use, for some authors, of other diagnostic approaches, including those based on the quantification of the number of leukocytes in the CSF [6,7]. Thus, a cell count ≥50 cells / mm^3 is generally accepted as the stigma of relapse, even if no threshold is consensually established at present in the medical literature [6,8]. However, the increase of leukocytes in CSF cannot consist alone the pathognomonic sign of a relapse of the THA, it occurs in other clinical situations related to various germs. The difficulty to diagnose a relapse in the HAT leads many authors to adopt serological and molecular approaches. These techniques are far from bringing consensus in the management of the post-treatment phase because of result

interpretation issue [9,10,11]. The limitations relate in particular to the current knowledge of the kinetics of the antibody anti-trypanosomes during the post-treatment period or to the duration of the DNA latency of the trypanosomes yet destroyed by effective trypanocidal treatment [10]. Before total clearance of nucleic acids, false positive cases can be diagnosed, showing the need for some teams to use the immune trypanolyse [11]. Regarding the immunological approach, the situation of the CATT-positive subjects, but negative for parasitological analysis, constitutes a challenge for the diagnosis and therapeutic management of the HAT to *T.b.gambiense*. A survey in Ivory Coast highlighted this gap, suggesting the interest of innovative approaches such as molecular biology and gene sequencing which are little or no accessible in most endemic countries [8]. Some previous studies have shown, however, discrepancies between the results of serological tests and those obtained by molecular methods [9].

This justifies the development of alternative diagnostic tools which are more operational, especially in highly endemic countries but also the poorest. Previous work by our team, describing the problem of diagnosis of relapse post-treatment phase of the HAT to *T.b.gambiense*, already suggested the potential value of a score from a multivariate analysis of a combination of 16 biomarkers, releasing the power of association of VS and blood eosinophilia [12].

This investigation aims to determine the interest of some simple clinical and biological parameters and easily accessible, in discrimination between relapse and non-relapse in patients with post-treatment phase with sterile CSF parasitological analysis but having abnormal neurological and psychological manifestations.

Patients and Methods

This cross-sectional study compares two groups of patients followed at the National Human African Trypanosomiasis Reference Center (CNRTHA) at Kinshasa (DR Congo) during the period from January 2004 to December 2008.

Inclusion criteria

Were included in this study, patient records that meet the criteria below:

- Having in its history evidence of a stage-2 HAT, parasitologically confirmed by the presence of trypanosomes in the CSF;
- Having received a full cure trypanocide which is effective for the nervous stage of HAT, including melarsoprol or Difluoro-Methyl-Ornithine (DFMO);
- Having been compulsorily examined by parasitological method as a test control for the absence of the parasite in CSF at the end of treatment with confirmation of sterilization;
- Having after a period of remission, redeveloped various neuropsychiatric disorders, CSF is sterile for direct parasitological examination.

The patients were then divided into group 1 (relapse) versus group 2 (not relapse), depending on the diagnosis retained by the medical team selected at the end of each file by the medical team. This diagnosis was confirmed by the response to trypanocides prescribed for a second time.

Statistical analyses

A filled and validated form allowed collecting data which have been processed in a micro-computer hp and managed with the help of the software Microsoft Office Excel 2007. Quantitative data are expressed in absolute value with SD (standard deviation) and qualitative ones in frequency (%).

With SPSS software version 12.0 and 16.0, clinical and biological parameters of interest were compared between the two groups, using the Fischer, chi-square of Pearson or the U Mann-Whitney tests accordingly to the cases, as well as with a score reported elsewhere by our team [12]. The 5% threshold was adopted to declare the meaning of the results ($p \leq 0.05$).

Operational definitions

Cytological and bio chemistry profile of CSF:

- These are based on quantitative variations in the number of leukocytes and protein levels in CSF.
- It is normal for a leukocyte count of 0-5 elements/mm^3 and a protein level of 10-45 mg%, according to laboratory standards of RNCHAT.
- Meningitis type, during simultaneous increase in leukocytes and protein level.
- Albumino-cytological dissociation type, when the increased protein level is associated with a normal cell count.
- Cyto-albumin dissociation type, when an increased cell count is combined with a normal protein concentration level.

Mode of onset of symptoms

Sudden onset: characterized by the occurrence of acute symptomatology without warning prodromal clinical manifestations.

Gradual appearance: characterized by a symptomatology in which the status phase is realized after a delay of at least 1month of clinical evolution.

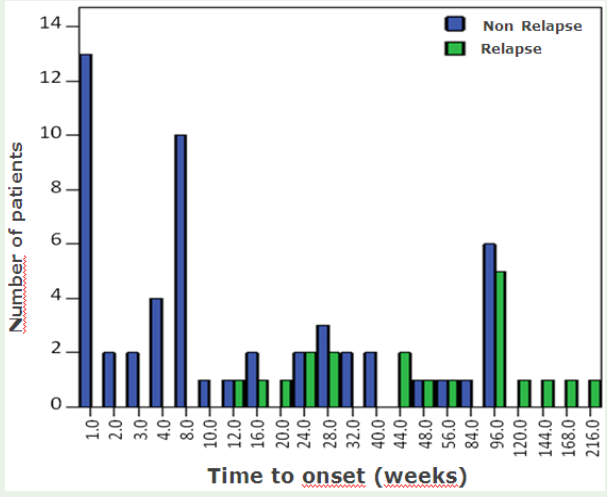

Figure 1: Time to onset of symptoms Group-1 *vs* Group-2.

Table1: Details of symptoms onset.

Type	Relapse (1)	Non relapse (2)	Total	%
Brutal	1	32	33	45,2
Progressive	14	17	31	42,5
No information	5	4	9	12,3
Total	20	53	73	100

The symptoms occurred gradually in the relapse whereas in the non-relapse the onset is of acute form (0.015).

Clinical latency period: Interval of time between the end of the treatment with trypanocide and the time of appearance of new clinical manifestations.

Relapse or Re-Infection: In this work, this concept appoints lack of molecular evidence, all neuropsychiatric clinical signs of favorable outcome under trypanocide treatment of second intention in the absence of any other disorder of the central nervous system.

No-Relapse: Patients with poor therapeutic response to second-line trypanocide drugs.

Results

Clinical latency period

The diagram below illustrates the differences in the time to onset of symptoms in the first post treatment period between the two groups (relapse (1) *vs* no relapse (2). (Figure1)

Relapse occurred after a clinical latency period ≥12 weeks; while in case of no relapse, clinical manifestations were early onset (≤12weeks) and late (>12weeks) (p=0.000).

Types of symptoms onset

The terms of symptoms occurrence are presented in (Table 1).

Data analysis grouped into major syndromes (not shown in the text) revealed no differences between the two groups (p≥0,233). However, individual signs including: epileptic, confusion, coma, schizophrenia, choreo-athetoid, intracranial hypertension, neurogenic device cordonal posterior and acute delirious, have been encountered in the group-2.

Table 2: Cytology and biochemistry of CFS (mean).

Parameter of CSF	Statistics	Relapse	Non relapse	P
Elements (/mm³)	Mean	199,80	40,94	0,000
	SD	187,56	79,68	
	Median	122,25	4,54	
	Minimum	6	1	
	Maximum	720	390	
Lymphocytes (/mm³)	Mean	121,17	24	0,000
	SD	121,45	54,29	
	Median	80,00	3,5	
	Minimum	5	1	
	Maximum	420	300	
Neutrophiles (/mm³)	Mean	53,76	9,30	0,001
	SD	78,21	18,83	
	Median	28,3	1,38	
	Minimum	0	0	
	Maximum	300	90	
Proteins(mg%)	Mean	85,68	64,30	0,001
	SD	26,62	19,71	
	Median	78,75	60,51	
	Minimum	60	40	
	Maximum	150	120	

Table 2 shows that the average rate of leucocytes and CSF proteins are increased in the relapse (p≤0.001).

Table 3: Shows the distribution of different CSF profiles.

CSF Profile	Relapse	Non-Relapse	Total	P
Meningitis	20	15	35	0,000
Dissociation Albumino-Cytological	0	24	24	
Normal	0	13	13	
Dissociation Cyto-Albumin	0	1	1	
Total	20	53	73	

Four cyto-biochemistry profiles of CSF were identified. Relapse is associated with meningitis profile (20/20 cases); while in the non-relapse, variable profiles were found with a predominance of albumino-cytological dissociation profile (p=0.000).

Biological data

Biological data include the complete blood count in addition to cyto-chemistry analysis of CSF.

The blood count showed no significant difference between the 2 groups (p≥0.467).

Anemia and elevated ESR were common in both groups.

Some CSF parameters, however, revealed striking differences (p≤0,001), defining cyto-chemistry profiles more or less specific between the two groups (Tables 2 and 3).

Four cyto-biochemistry profiles of CSF were identified. Relapse is associated with meningitis profile (20/20 cases); while in the non-relapse, variable profiles were found with a predominance of albumino-cytological dissociation profile (p=0.000).

Score

(Table 4) shows a score>0 in almost all subjects in group-1 (12/13 cases; 92.3%); but no significant difference for group 2 (10 *vs* 7 cases).

Discussion

This investigation aimed to determine some simple and accessible bio-clinical markers of discrimination between relapse versus non relapse during the post-treatment phase of HAT stage-2.

Relapse seems more plausible when clinical symptoms settle gradually, after a latency of at least 3months. The profile of the CSF is usually meningitis type with a hyperleucocytorachy (≥120 cells/mm³) and a score>0.

The non relapse is more likely to have symptoms of more acute onset after a variable latency period associated with a CSF of albumino-cytological dissociation profile type, with normal leukocytes count ≤5 elements/mm³ and often a score <0.

The discriminative nature of the duration of clinical latency period (≥12 weeks) and the modality of gradual onset of symptoms to relapse are in agreement with previous experimental investigation by MULUMBA and al on rats [2]. In fact, these authors described a proven biological relapse in a murine model with a clinical latency greater than 12 weeks. These authors justified this time by the adaptation phase necessary for adapting the trypanosome in the

Table 4: Score distribution calculated between the two groups.

0	Relapse	Non relapse	Total	P
> 0	12	10	22	0,005
< 0	1	7	8	
TOTAL	**13**	**17**	**30**	

Table 4 shows a score > 0 in almost all subjects in group 1 (12/13 cases; 92.3%); but no significant difference for group-2 (10 *vs* 7 cases).

environment. This observation, however, requires taking into account the species barrier before any extrapolation.

In a clinical equivalence trial in Angola including 500 subjects treated with Arsobal, the authors reported a variability of clinical latency between 1 to 24 months [13]. In Ivory Coast, the similar research in a longitudinal survey which involved 812 sleeping sickness in stage-2 after treatment with Melarsoprol revealed that the latency period is between 3 and 18 months [8]. Other previous studies have even described latency periods longer than 10 years before relapse of the HAT [14]. These observations according to the of latency period are in agreement with our report; and a latency period of at least 3 months-appears rather strongly associated with relapse, apart from the phenomenon of trypanotolerance [7]. MULUMBA and al showed that the period of at least 3 months of latency was required for maximum growth of the parasite before the onset of clinical manifestations meningoencephalitic manifestations [2]. The greatest variability of the duration of the clinical latency period in the non relapse group, suggests the possibility of other phenotypes to be addressed.

The onset of symptoms was gradual in relapse and rather brutal in non relapse in this investigation. The progressive onset is related to the life cycle of *T.b.gambiense* in the vertebrate host as mentioned elsewhere; this parasite usually determines a gradual onset symptoms and a chronic progressive disease.

Clinical manifestations described in this study are those reported by several authors [15,16,17,18]. Day-time sleepiness with or without inversion of circadian rhythm and the frontal syndrome rank well among the most frequent manifestations. The predominant topography of pathological lesions in the frontal lobes would be one explanation [19].

The compared biological data revealed an increase of the protein level as well as the leukocytes count in the CSF for relapse. This situation is commonly described in the subacute *T.b.gambiense* meningoencephalitis [15,17,18]. The presence of the parasite in the brain parenchyma could induce inflammatory reactions leading to the increase of the elements and the protein observed.

DJE and al have designated this phenomenon "biological syndrome relapses" [20]. The median value of leukocytes in CSF of 122.25 cells/mm^3 in relapse reported here, is in agreement with those in other previous work from DR Congo and elsewhere [6-8,17,21]. MUMBA et al have found in 63 patients with relapse of HAT in our country, a CSF leukocyte count ≥50 cells/mm^3, in an assessment of the performance of several diagnostic criteria [6].

MIEZAN and his team described a leukocyte counts in order of 158-185 in 32 patients in confirmed relapse out of 812 treated with Melarsoprol in the focus of Daloa, Ivory Coast [8]. In a review of the literature on post-therapeutic profile of leukocytes in CSF in HAT, LEJON and BUSCHER concluded that relapse was always associated with a leukocyte count higher than 50 cells/mm^3 [21].

The increase in leukocyte levels in CSF is an immunological phenomenon related to an early recruitment of leukocytes into the CSF, a preferred site in Trypanosoma infection [22,23]. The kinetics of inflammatory cells is controlled by various mediators produced by astrocytes chemotactic activated during parasitic invasion. The increase of the CSF leukocyte count can be found in other infection than in trypanosomiasis; it is far to represent the only reliable criterion for relapse diagnosis in this disease. Reason why the interest to seek other alternatives for better orientation and therapeutic decision. In this context, MIEZAN and al already advocated the association of antitrypanosoma antibody in CSF, neurological signs and the cyto-biochemistry CSF profile [8].

The meningitis cyto-biochemistry profile of the CSF observed in relapse is in agreement with other previous reports [21-23]. In the non-relapse, the CSF cyto-biochemistry profile is most frequently of albumino-cytological dissociation type. Apart from all spinal cord compression syndrome, this pattern suggests the involvement of immune mechanisms as in certain neuro-immune diseases conventionally known, including GUILLAIN-BARRE syndrome, Multiple Sclerosis (MS), Subacute Sclerosingpanencephalitis (SSPE) or VAN BOGAERT disease. The increasing CSF protein count should be due to intrathecal production, demonstrating the importance of inflammatory reactions.

The observations reported in this study, however, need to be interpreted with caution. The first limitation of the investigation is the very concept of relapse versus non relapse which was based on the appreciation of the medical staff of the center, without any traceability by molecular analyzes. The second limitation is the lack of performance of statistical tests including sensitivity, specificity, ROC curve, due to the lack of standard gold for comparison which is the molecular biology. The third limitation is the inability to extrapolate from single-center results into the situation of all HAT sites [24]. Finally, the score calculated and discussed in this work is based on a small number of cases and deserves even greater validation. However, these limits do not empty the work of all its interest. It is one of the first to determine accessible diagnostic criteria that could be spreaded. This study lays the framework for broader investigations and operational research in our context.

Conclusion

Neuropsychiatric disorders in post-treatment period of HAT raise a real problem of diagnosis in patients with sterile CSF by direct parasitological examination. The present study shows the interest of some clinical markers and CSF cyto-biochemistry analyzes in discrimination between relapse and non-relapse. Studies including a larger number of subjects and comparing our data with that of molecular biology will contribute to a better validation of the observations.

References

1. Olivier G, Legros D. Trypanosomiase Humaine Africaine: Historique De La Thérapeutique Et De Ses Echecs. Tropicalmedicine and International Health. 2001; 6: 855-863.
2. Mulumba MP. Dynamique De Développement De Trypanosoma Brucei Gambiense Dans Les Tissus De L'hôte Vertébré: Aspects Parasitologiques, Thérapeutiques Et Pronostiques. Thèse Doctorale, Universitaire Instelling Antwerpen, Departement Geneskunde. 1984.
3. Poltera AA. Cryptic Stage Of Sleeping Sickness Trypanosome Developing in Choroid Plexus Epithelial Cells. British Medical Journal. 1983; 286: 52-53.
4. Jennings FW, Whitelaw DD, Holmes PH, Chizyuka HGB, Urqu-Hart GM. The Brain is a Source of Relapsing Trypanosoma Brucei infection in Mice after Chemotherapy. International Journal of Parasitology. 1979; 9: 381-384.
5. Wolburg H, Mogk S, Acker S, Frey C, Meiner TM. Late Stage Infection in Sleeping Sickness. PLOS ONE. 2012; 7: e34304.

6. MumbaNgoyi D, Lejon V, N'siesi FX, Boelaert M, Buscher P. Comparison of Operational Criteria for treatment Outcome in Gambiense Human African Trypanosomiasis. Tropical Medicine and International Health. 2009; 14: 438-444.

7. Lejon V, Bentivoglio M, Franco Jr. Human African Trypanosomiasis. Handbook of Clinical Neurology. 2013; 114: 169-181.

8. Miezan TW, Dje NN, Doua F, Boa F. Trypanosomose Humaine Africaine En Côte d'Ivoire: Caractéristiques Biologiques Après Traitement. A Propos De 812 Cas Traités Dans Le Foyer De Daloa (Côte d'Ivoire) Bulletin De La Société De Pathologies Exotiques. 2003; 95: 362-365.

9. Garcia A, Jamonneau V, Magnus E. Follow-Up of Card Agglutination Trypanosomiasis Test (CATT) Positive but Apparently Aparasitemic Individuals in Côte d'Ivoire: Evidence for Complex and Heterogenous Population. Tropical Medicine and International Health. 2000; 5: 786-793.

10. Jamonneau V, Solano Ph, Koffi M, Dezinot M, Cuny G. Apport Et Limites Du Diagnostic De La Trypanosomiase Humaine Africaine. Médecine Et Sciences. 2004; 20: 871-875.

11. Jamonneau V, Bucheton B, Kabore J, Ilboubo H, Camara O, Courtin F, et al. Revisiting the Immune Trypanolysis Test to Optimize Epidemiological Surveillance and Control of Sleeping Sickness in West Africa. Plos Neglected Tropical Diseases. 2010; 4: e917.

12. Itakala B, Mulumba MP, Kayembe K, Lombe N, Tshala K. Problématique Du Diagnostic De Rechute Dans Le Traitement De La Trypanosomiase Humaine Africaine À Trypanosoma Brucei Gambiense En Pratique Hospitalière. Congo Médical. 1998; 2: 434-442.

13. Schmid C, Nkunku S, Merolle A, Vounatsou P, Burri C. Efficacy of 10-Day Melarsoprol Schedule 2 Years after Treatment for Late-Stage Gambiense Sleeping Sickness. Lancet. 2004; 364: 789-790.

14. Gentilini M, Duflo B. Médecine Tropicale. 4ème Edition, Flammarion, Paris, 1986; 839.

15. Edan G. Signes Cliniques Et Biologiques Des Trypanosomiases À T. Gambiense Vues Au Stade D'atteinte Méningoencéphalitique. Médecine Tropicale. 1979; 39: 499-507.

16. Dumas M, Bisser S. Clinical Aspects of Human African Trypanosomiasis. in: Progress in Human African Trypanosomiasis, Sleeping Sickness (Dumas, M., Bouteille, B. &Buguet, A.) Ed.Springer, Paris. 1999; 215-233.

17. Burri C, Brun R. Human African Trypanosomiasis. In: Manson's Tropical Diseases 21st Ed. (Eds Cook GC &Zumla AI) Elsevier, Edinburgh. 2003; 1303-1323.

18. Brun R, Blum J, Chappuis F, Burri C. Human African Trypanosomiasis. The Lancet. 2010; 275: 148-159.

19. Adams JH, Hallier L. A Neuropathological Analysis of Eight Fatal Cases of African Trypanosomiasis (T.B. Gambiense) Neuropathology and Applied Neurobiology. 1984; 10: 304-305.

20. DJÈ N.N. La Qualité De La Vie Après traitement De La T.H.A. Etude Prospective Sur 424 Patients Traités Et Suivis Au P.R.C.T. De Daloa. Thèse De Médecine, Abidjan, 1997.

21. Hatz C, Brun R. Zerebraletrypanosomose: Aspekte in Epidemiologie, Klinik, Therapie Und Krankheitsverlanf. Schweiz. Med. Wochensche. 1992; 122: 873-878.

22. Courtioux B, Oda C, Vatunga G, Pervieux L, Joseenando T, M'eyi Pm, et al. A Link between Chemokine Levels and Disease Severity in Human African Trypanosomiasis. International Journal of Parasitology. 2006; 36: 1057-1065.

23. Courtin D, Jamonneau V, Mathieu JF, Koffi M, Millet J, Yeminanga CS, et al. Comparison of Cytokine Plasma Levels in Human African Trypanosomiasis. Tropical Medicine and International Health. 2006; 11: 647-653.

24. Itakala Fb, Mananga Gl, Situakibanza Hn, Mumba DN, Nengi Net, Muyembe Jt. Neuropsychiatric Disorders Following the Treatment of Second Phase of Human African Trypanosomiasis. Annales Africaines De Medecine. 2015; 8: 2027-2033.

Citation: Itakala FB, Kayembe JMNT, Mananga Gl and Mashinda DK. Relapse Versus Non Relapse in Human African Trypanosomiasis: Simple Criteria for Discrimination. SM Journal of Infect Dis. 2016; 1(1): 1003.