Treatment failure in hemo-lymphatic stage sleeping sickness patients is related to intrathecal IgM synthesis, cerebrospinal fluid IgM and interleukin-10

Running title: Markers of CNS involvement in HAT

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Abstract

Human African trypanosomiasis treatment is stage-dependent, but staging is controversial. Central nervous system involvement and its relationship with suramin treatment failure were assessed in 60 patients with parasitologically confirmed *Trypanosoma brucei (T.b.) gambiense* infection in hemo-lymphatic stage (white blood cell count ≤ 5/µl and no trypanosomes in the CSF). The prognostic value of cerebrospinal fluid (CSF) interleukin-10, IgM (by nephelometry and point-of-care LATEX/IgM test), total protein and trypanosome specific antibodies was assessed. IgM and interleukin-10 were measured in serum and the presence of neurological signs, intrathecal IgM synthesis and blood-CSF barrier dysfunction was determined. After suramin treatment, 14 out of 60 patients relapsed (23%). Relapses were significantly correlated with intrathecal IgM synthesis (OR 46; 95% CI 8 to 260), CSF IgM ≥ 1.9 mg/l (OR 11.7; 95% CI 2.7 to 50), CSF end titer in LATEX/IgM ≥ 2 (OR 10.4; 95% CI 2.5 to 44) and CSF interleukin-10 > 10 pg/ml (OR 5; 95%CI 1.3 to 20). Sensitivity of these markers for treatment failure was 43 to 79% and specificity was 74 to 93%. The results show that *T.b. gambiense* patients with signs of neuro-inflammation in CSF, who are treated with hemo-lymphatic stage drugs, are at risk of treatment failure. This highlights the need for development and evaluation of accurate point-of-care tests for staging of human African trypanosomiasis.

The authors declare not to have a commercial or other association that might pose a conflict of interest.

Parts of the results have been presented as a poster at the 28th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Addis Abeba, Ethiopia, 26-30/9/2005.

Introduction

Infection with the parasites *Trypanosoma brucei* (*T.b.*) *gambiense* and *rhodesiense* causes human African trypanosomiasis (HAT) or sleeping sickness. The disease progresses from a first hemo-lymphatic stage towards a second meningo-encephalitic stage as trypanosomes invade the brain (22). Brain invasion may occur after weeks in the acute *rhodesiense* disease form, and after months or years in chronic *T.b. gambiense* infection. Drugs for treatment of the first hemo-lymphatic disease stage, pentamidine and suramin, are relatively safe but ineffective in the second, meningo-encephalitic stage. The latter is routinely treated with Melarsoprol, or with IV eflornithine (21). Melarsoprol is a toxic drug associated with up to 5% of fatal encephalitic reactions and is therefore not recommended for hemo-lymphatic stage treatment. Since there are no specific clinical or biochemical signs in blood indicating the onset of the meningo-encephalitic stage, staging and treatment choice are based on examination of the cerebrospinal fluid (CSF). White blood cell (WBC) counts > 5/µl or presence of trypanosomes in the CSF are routinely used as markers for central nervous system (CNS) involvement (22). Unfortunately, the limited specificity and sensitivity of these tests may lead to incorrect staging of the disease with false positive and false negative interpretations, and subsequently inefficient or unnecessarily toxic treatment.

Detection of intrathecal IgM synthesis is a specific and sensitive parameter to detect CNS involvement in HAT caused by *T.b. gambiense* (1), but the

technology to assess intrathecal synthesis is not suitable for field use. As a consequence of intrathecal synthesis, levels of IgM and trypanosome-specific antibodies are increased in the CSF of meningo-encephalitic stage patients in addition to blood derived fractions (7,13). Two point-of-care tests were developed on this basis: the LATEX/IgM, for IgM detection in the CSF of sleeping sickness patients (10), and the LATEX/*T.b. gambiense*, for detection of trypanosome-specific antibodies (4). Both are self-contained tests that can be performed out of the laboratory, need a minimum of equipment and give immediate results. Interleukin-10 (IL-10) has also been proposed as a potential marker for stage determination, because high serum and CSF IL-10 concentrations have been observed in meningo-encephalitic stage patients, and those concentrations decline quickly after treatment (9,14).

Intrathecal IgM synthesis, CSF IL-10, CSF IgM, the LATEX/IgM and the LATEX/*T.b. gambiense* were assessed as markers of CNS involvement in hemo-lymphatic stage *T.b. gambiense* patients. Their relationship to treatment failure was studied.

Methods

Patients and samples

Patients were consecutively enrolled during the routine screening and treatment procedures of the HAT control programme at Bwamanda hospital (Equateur Province, D.R. Congo) between February and April 1998. Patients with parasitologically confirmed *T.b. gambiense* infection in hemo-lymphatic stage (CSF WBC count ≤ 5/µl and no trypanosomes in the CSF after double centrifugation) were eligible for this study. Patients with hemorrhagic CSF were excluded. All patients underwent a complete clinical and neurological examination for the detection of sleep disturbances, primitive and deep tendon reflexes, mental state alterations, abnormal movements, convulsions, sensory neuropathy, and anomalies of tone and coordination. After pre-treatment for co-infections (malaria, helminthiasis), they were treated with suramin (6 IV injections of 20 mg/kg, 3 days of rest between each injection). Serum and CSF samples were taken for routine diagnostic purposes before treatment, 24 hours and 3, 6, 12, 18 and 24 months post-treatment when patients underwent a full clinical, parasitological and CSF examination. Remaining volumes of serum and CSF were frozen until analysis. Treatment failure (or relapse) was defined as i) reappearance of trypanosomes in serum or CSF during follow-up , ii) a combination of CSF WBC count > 20/µl and worsened general condition during follow-up, and/or iii) a CSF WBC count > 20/µl after 24 months of follow-up. The increased cut-off for relapse of 20 WBC/µl was chosen to avoid unnecessary

113 retreatment (2,16). Treatment failures were given rescue treatment with second-
114 line regimens (2). From each patient or accompanying relative, informed consent
115 was obtained prior to enrolment in the study. The study was approved by the
116 National Ethical Committee of the Ministry of Health of D.R. Congo.
117
118 Analyses
119 IgM and albumin in CSF and serum were quantified on a nephelometer (Prospec;
120 Dade-Behring). Blood-CSF barrier function was evaluated using the CSF/serum
121 albumin quotient, Q_{Alb}, with upper reference limit $Q_{Alb}=(4+age/15)x10^{-3}$ (19). The
122 intrathecal IgM fraction was $IgM_{IF}=(1-Q_{Lim}(IgM)/Q_{IgM})x100$, with $Q_{IgM}=$
123 CSF/serum IgM quotient, and $Q_{Lim}(IgM)=0.67x(Q_{Alb}^{2}+120x10^{-6})^{1/2}-7.1x10^{-3}$ (19).
124 An intrathecal fraction $IgM_{IF} > 0\%$, was considered positive for intrathecal IgM
125 synthesis.
126 For IgM quantification in CSF using LATEX/IgM (10), twenty µl of LATEX/IgM
127 reagent were mixed with 20 µl of sample on a test card. The card was rocked
128 and the agglutination was scored after 5 minutes. The end titre of a sample -the
129 highest dilution yielding agglutination- was determined.
130 Specific antibodies in CSF were detected by mixing 15 µl of LATEX/*T.b.*
131 *gambiense* with 30 µl of CSF on a test card (4). Patients with reaction of
132 undiluted CSF in LATEX/*T.b. gambiense* after 10 minutes rocking were
133 considered positive.
134 Interleukin-10 was quantified in a sandwich ELISA using unlabeled and
135 biotinylated rat anti-human IL-10 (BD Biosciences, Pharmingen) as capturing and

detecting antibody. Serial twofold dilutions of serum (1:4-1:16) and CSF (1:2-1:8) were tested. In each plate a standard curve of 0-100 pg/ml recombinant human IL-10 (National Institute for Biological Standards and Control, UK) was included. Streptavidin-peroxidase polymer ultrasensitive (Sigma, Belgium) was used as conjugate. The reaction was revealed using ABTS (Boehringer, Germany) and the optical density read at 415 nm (Multiskan RC Version 6.0, Labsystems). Detection limits were 6.7 and 13.4 pg/ml IL-10 for CSF and serum.

The total CSF protein concentration was determined in duplicate with the bicinchoninic acid protein assay using the microtiter plate protocol (Pierce, Rockford, IL) with bovine serum albumin as standard. All these analyses were performed after completion of the follow-up.

Data analysis

Median and inter-quartile range were computed for each test. Median values for relapsing and non-relapsing patients were compared using the Kruskal-Wallis test. Continuous variables were dichotomised and compared with the Fisher exact test. Odds ratios with a 95% Confidence Interval (CI) were calculated to assess the association with relapse. Sensitivity, specificity, positive and negative predictive value were calculated with a 95% CI. Receiver-operator characteristics (ROC) curves were constructed to compare sensitivity and specificity of each parameter for detection of relapse over the whole spectrum of possible cut-off values. The area under the curve (AUC) was calculated to

158 quantify overall diagnostic accuracy (6,23). Data were analysed using SAS ©
159 and Stata 8 ©.

Results

An overview of the study enrolment and follow-up process is shown in figure 1. Among 73 enrolled patients thirteen patients could not be included in the analysis for the following reasons. Two patients died before or during treatment of bronchopneumonia and pneumococcal meningitis. No pre-treatment samples were kept for further analyses of 3 patients, 3 patients left the centre before the 24 hour follow up time point and 5 patients failed to attend for any other follow-up visits. The analysis reports on 60 patients, out of whom 14 relapsed (23.3%). The follow-up period (last follow-up visit after treatment) was $\geq$ 24 months for 33, 18 months for 8, 12 months for 6, 6 months for 5 and 3 months for 8 patients. The male/female ratio was 1/2, the mean age 32.9 years $\pm$ 10.1 (range 12-65).

Pre-treatment CSF and serum parameters are given in table 1. A significant difference was observed between cured and relapsed patients for the intrathecal IgM fraction, the CSF IgM concentration, LATEX/IgM end titer and IL-10 concentration.

There was a significant association between the occurrence of relapse and presence of intrathecal IgM synthesis before treatment (table 2). The high diagnostic accuracy of IgM_{IF} was reflected by the AUC of 0.86 (95% CI 0.73-0.98). Presence of intrathecal IgM synthesis (IgM_{IF} > 0%) had a sensitivity of 76.9% (66.3-87.6) and specificity of 93.2% (81.3-98.6) for relapse. Positive and negative predictive values were respectively 76.9% (66.3-87.6) and 93.2% (81.3-98.6).

For the CSF IgM concentration, the AUC was 0.81 (0.69-0.92). The highest diagnostic accuracy was obtained using a cut-off of 1.9 mg/l, combining a sensitivity of 78.6% (68.2-89.0) with a specificity of 76.1% (65.3-86.9). An IgM concentration in CSF higher than this cut-off was associated with relapse (table 2). Positive and negative predictive values were 50.0% (28.2-71.8) and 92.1% (78.6-98.3).

Cerebrospinal fluid LATEX/IgM end titers ≥ 2 were associated with relapse. The AUC for the CSF LATEX/IgM end titer was 0.81 (0.68-0.93). A cut-off of CSF LATEX/IgM end titer ≥ 2 had a diagnostic sensitivity of 78.6% (49.2-95.3) and specificity of 73.9% (58.9-85.7) and positive and negative predictive values of 47.8% (26.8-69.4) and 91.9% (78.1-98.3).

Interleukin-10 concentrations in CSF above 10 pg/ml were significantly associated with relapse. The AUC was 0.70 (0.52-0.88). The diagnostic accuracy for relapse when IL-10 is > 10 pg/ml was 42.9% sensitivity (30.3-55.4) and 87.0% specificity (78.4-95.5). Positive and negative predictive values were respectively 50.0% (21.1-78.9) and 83.3% (69.8-92.5).

We observed no detectable relationship of the CSF total protein concentration, the serum IgM concentration nor the serum IL-10 concentration with relapse. The AUC for these parameters were respectively 0.49, 0.55 and 0.63. Presence of blood-CSF barrier dysfunction (in 25% of cured and 31% of relapsed patients) nor positivity of CSF in LATEX/*T.b. gambiense* (in 4% of cured and 14% of relapsed patients) were significantly associated with relapse.

The most common neurological abnormalities were sleep disturbances (20/60 patients, 30%), presence of primitive and deep tendon reflexes (13% and 12%), and confusion (6%). However, none of those neurological signs was significantly associated with relapse in the patients studied.

Discussion

We demonstrate that presence of intrathecal IgM synthesis, elevated CSF IgM and elevated CSF IL-10 concentrations are significantly associated with failure of suramin treatment in hemo-lymphatic *T.b. gambiense* patients.

Intrathecal IgM synthesis, detected as intrathecal fraction IgM_{IF}, was previously shown to be the most sensitive marker for CNS involvement in *T.b. gambiense* patients (12). It was present in 22% of patients in this study, which corroborates earlier reports of intrathecal IgM synthesis in up to 14 % of first stage patients (1,12). Previously we also showed that presence of intrathecal IgM synthesis in early second stage patients treated with pentamidine, is associated with risk for treatment failure (11). We now demonstrate a similar association in first stage patients treated with suramin. Detection of intrathecal IgM synthesis had the best sensitivity and specificity for relapse, and a high positive and negative predictive value.

Unfortunately, in HAT-endemic areas, IgM_{IF} determination is not possible in rural health centres that are not equipped for quantitation of albumin and IgM. The high IgM concentrations in CSF of sleeping sickness patients originate both from an increased blood derived fraction and from intrathecal synthesis, and are detectable by LATEX/IgM (10,12). Indeed, IgM_{IF}, the total CSF IgM concentration and the CSF LATEX/IgM end titer were correlated (data not shown). Elevated CSF IgM concentrations $\geq$ 1.9 mg/l and CSF LATEX/IgM end titers $\geq$ 2 were associated with treatment failure and had acceptable sensitivities

of 79% and specificities of 74-76% for relapse. The LATEX/IgM end titer cut-off of ≥ 2 is lower than the cut-offs of 4 or 8 proposed before (10,11). The serum and CSF IgM concentrations in these first stage patients were also lower than those reported earlier (12). Such unexplained variation shows that proposing cut-offs for IgM in CSF for disease staging in HAT is a complex issue. The absolute value of CSF IgM depends on the serum IgM concentration and barrier function (12) in contrast to IgM_{IF}, which takes these aspects into account (19). The lower cut-off in the present group might therefore be a consequence of lower blood derived concentrations, due to lower blood IgM concentrations and a lower frequency of blood-CSF barrier dysfunction. Despite this shortcoming, LATEX/IgM is the only test suitable for field use amongst all those examined. LATEX/IgM, or other point-of-care tests for IgM detection in CSF merit further validation.

Although its AUC, sensitivity and specificity were inferior to those of the IgM associated parameters, a relationship between the CSF interleukin-10 concentration and treatment failure was demonstrated for the first time. The observed IL-10 concentrations overlap with those reported previously for *T.b. gambiense* and *rhodesiense* first stage patients (9,14). None of our patients showed higher IL-10 concentrations in CSF than in serum (data not shown), which does not exclude the intrathecal origin of this cytokine in CSF (15).

In contrast with our findings in early second stage patients (11), no association between trypanosome specific antibodies in CSF and treatment failure was observed. Absence of any detectable relationship between the total CSF protein

concentration and relapse confirms earlier findings (11) and the limited impact of CSF total protein determination for disease staging (12). The irrelevance of clinical signs and symptoms for staging of human African trypanosomiasis was corroborated (3). Absence of a detectable relationship between serum IL-10 or IgM and relapse is not surprising as trypanosomiasis infection induces a strong immune stimulation in the hemo-lymphatic compartment.

Although pentamidine is the first-line treatment for *T.b. gambiense* patients in hemo-lymphatic stage, patients in this study were treated with suramin because of a shortage in the supply of pentamidine. Published suramin cure rates in *T.b. rhodesiense* and *gambiense* patients are around 95% (18). The observed relapse rate with suramin of 23% therefore seems high, but corresponds to the failure rates between 25 and 35% reported for suramin treatment of first stage *T.b. gambiense* patients in D.R. Congo in the 1950s (17,18). Due to the unusual treatment regimen and its relatively high failure rate, results should be interpreted with care, especially when extrapolating to patients treated with pentamidine. However, our findings correspond to an earlier study carried out with pentamidine-treated patients (11). In addition, the relatively high positive predictive values around 75 and 50% of intrathecal IgM synthesis and CSF IgM and LATEX/IgM are influenced by the high relapse rate found in this study. Relapse rates below 10%, would result in lower positive predictive values.

We cannot exclude the possibility that our study is slightly biased as 22/60 (37%) of the patients had incomplete follow-up (no treatment failure and last follow-up visit between 3 and 18 months post-treatment). This compliance is comparable

with figures reported in several clinical trials (5,8,20). The assumption that such patients with unknown outcome are cured is in line with other reports (20). Removal of patients with unknown outcome from the statistical analysis, had no substantial influence on the results: intrathecal IgM synthesis, elevated CSF IgM >1.9 mg/l, CSF LATEX/IgM end titers $\geq$ 2 and CSF IL-10 concentrations > 10 pg/ml remained significantly associated with treatment failure (data not shown).

Our findings lead us to question current staging in HAT. IgM and IL-10 in CSF are typical components of the neuro-inflammatory response in meningo-encephalitic stage *T.b. gambiense* patients. Although the exact biological role and significance of elevated IgM and IL-10 concentrations in CSF remain to be elucidated, our results illustrate the lack of accuracy of present staging tools, leading to misclassification and inappropriate treatment of cases. The urgent need for improved staging tools, preferentially point-of-care tests, is apparent. Prospective studies are needed to validate CSF IgM and CSF IL-10 as alternative staging tests and to assess how they can help clinicians to improve treatment decision.

Acknowledgements

Financial support was received from the Belgian Ministry of Foreign Affairs, Directorate General for Development Co-operation.

Logistic, medical and laboratory support was provided by the Centre de Développement Intégral-Bwamanda (CDI-Bwamanda, NGO); Medische Missie Samenwerking (MEMISA Belgium, NGO); the staff of Bwamanda hospital, in particular the late sister J. Verbunt, and the Programme National de Lutte contre la Trypanosomiase Humaine Africaine (PNLTHA, Ministry of Health, R.D. Congo). We also thank Dr. A. Nangouma (PNLTHA, Central African Republic) for help with transport of the samples, N. Bebronne (ITM, Antwerp) and K. Walther (University Göttingen) for help with analysis of the samples.

We do not have a commercial or other association that might pose a conflict of interest.

Reference List

1. **Bisser, S., V. Lejon, P. M. Preux, B. Bouteille, A. Stanghellini, M. O. Jauberteau, P. Büscher, and M. Dumas**. 2002. Blood-cerebrospinal fluid barrier and intrathecal immunoglobulins compared to field diagnosis of central nervous system involvement in sleeping sickness. J.Neurol.Sci. **193**:127-135.
2. **Bisser, S., F. X. N'Siesi, V. Lejon, P.-M. Preux, S. Van Nieuwenhove, M. M. C. Bilengue, and P. Büscher**. 2007. Equivalence trial of melarsoprol and nifurtimox monotherapy and combination therapy for the treatment of second-stage *Trypanosoma brucei gambiense* sleeping sickness. JID **195**:322-329.
3. **Boa, Y. F., M. A. Traore, F. Doua, M. T. Kouassi-Traore, B. E. Kouassi, and C. Giordano**. 1988. Les différents tableaux cliniques actuels de la trypanosomiase humaine Africaine à *T. b. gambiense*. Analyse de 300 dossiers du foyer de Daloa, Côte-d'Ivoire. Bull.Soc.Pathol.Exot.Fil. **81**:427-444.
4. **Büscher, P., V. Lejon, E. Magnus, and N. Van Meirvenne**. 1999. Improved latex agglutination test for detection of antibodies in serum and cerebrospinal fluid of *Trypanosoma brucei gambiense* infected patients. Acta Trop. **73**:11-20.

5. **Cross, P., F. Doua, and S. Jaffar**. 2006. The risk factors for relapse among patients with African trypanosomiasis in Daloa, Cote d'Ivoire. Trop.Doct. **36**:90-93.
6. **DeLong, E. R., D. M. DeLong, and D. L. Clarke-Pearson**. 1988. Comparing the areas under two or more correlated receiver operating curves: a non-parametric approach. Biometrics **44**:837-845.
7. **Greenwood, B. M. and H. C. Whittle**. 1973. Cerebrospinal-fluid IgM in patients with sleeping-sickness. Lancet **2**:525-527.
8. **Legros, D., S. Evans, F. Maiso, J. C. K. Enyaru, and D. Mbulamberi**. 1999. Risk factors for treatment failure after melarsoprol for *Trypanosoma brucei gambiense* trypanosomiasis in Uganda. Trans.R.Soc.Trop.Med.Hyg. **93**:439-442.
9. **Lejon, V., J. Lardon, G. Kenis, L. Pinoges, D. Legros, S. Bisser, X. N'Siesi, E. Bosmans, and P. Büscher**. 2002. Interleukin-6, IL-8 and IL-10 in serum and CSF of *T.b. gambiense* sleeping sickness patients before and after treatment. Trans.R.Soc.Trop.Med.Hyg. **96**:329-333.
10. **Lejon, V., D. Legros, M. Richer, J. A. Ruiz, V. Jamonneau, F. Doua, N. Djé, F. X. N'Siesi, S. Bisser, E. Magnus, I. Wouters, J. Konings, T. Vervoort, F. Sultan, and P. Büscher**. 2002. IgM quantification in the cerebrospinal fluid of sleeping sickness patients by a latex card agglutination test. Trop.Med.Int.Health **7**:685-692.
11. **Lejon, V., D. Legros, A. Savignoni, M. Gastellu Etchegorry, D. Mbulamberi, and P. Büscher**. 2003. Neuro-inflammatory risk factors for

353 treatment failure in "early second stage" sleeping sickness patients treated
354 with Pentamidine. J.Neuroimmunol. **144**:132-138.
355 12. **Lejon, V., H. Reiber, D. Legros, N. Djé, E. Magnus, I. Wouters, C. Sindic,**
356 **and P. Büscher**. 2003. Intrathecal immune response pattern for improved
357 diagnosis of central nervous system involvement in trypanosomiasis.
358 J.Infect.Dis. **187**:1475-1483.
359 13. **Lucasse, C.** 1964. Fluorescent antibody test as applied to cerebrospinal
360 fluid in human sleeping sickness. Bull.Soc.Pathol.Exot.Fil. **57**:283-292.
361 14. **MacLean, L., M. Odiit, and J. M. Sternberg**. 2001. Nitric oxide and
362 cytokine synthesis in human African trypanosomiasis. J.Infect.Dis.
363 **184**:1086-1090.
364 15. **MacLean, L., M. Odiit, and J. M. Sternberg**. 2006. Intrathecal cytokine
365 responses in *Trypanosoma brucei rhodesiense* sleeping sickness patients.
366 Trans.R.Soc.Trop.Med.Hyg. **100**:270-275.
367 16. **Miézan, T. W., N. N. Djé, F. Doua, and F. Boa**. 2002. Trypanosomose
368 humaine africaine en Côte d'Ivoire: caractéristiques biologiques après
369 traitement. A propos de 812 cas traités dans le foyer de Daloa (Côte
370 d'Ivoire). Bull.Soc.Pathol.Exot.Fil. **95**:362-365.
371 17. **Neujean, G.** 1950. Contribution a l'étude des liquides rachidiens et
372 céphaliques dans la maladie du sommeil a "Trypanosoma gambiense".
373 Ann.Soc.Belg.Méd.Trop. **30**:1125-1387.
374 18. **Pépin, J. and F. Milord**. 1994. The treatment of human African
375 trypanosomiasis. Adv.Parasitol. **33**:2-49.

19. **Reiber, H. and J. B. Peter**. 2001. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J.Neurol.Sci. **184**:101-122.

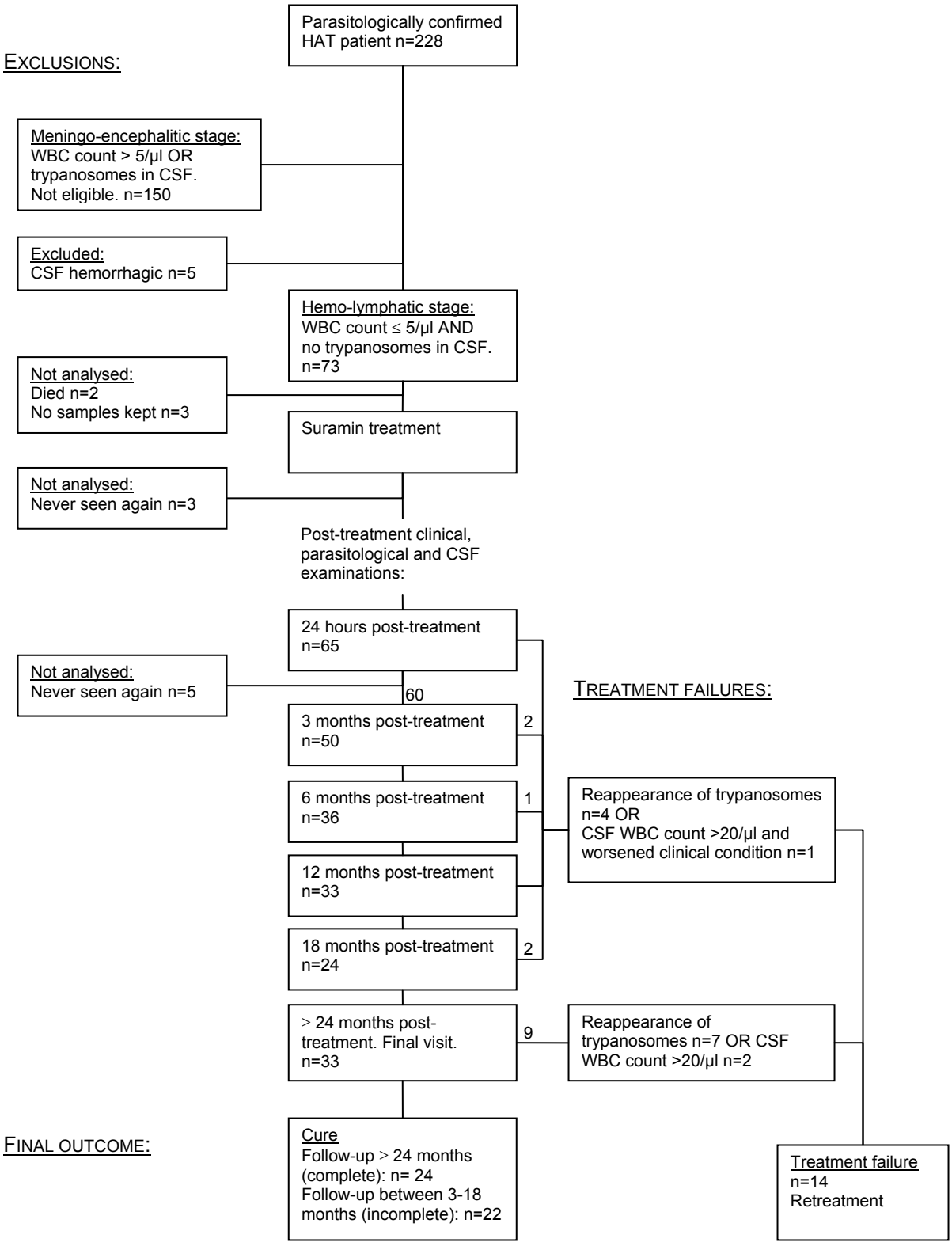
20. **Schmid, C., M. Richer, M. M. C. Bilengue, T. Josenando, F. Chappuis, C. R. Manthelot, A. Nangouma, F. Doua, P. N. Asumu, P. P. Simarro, and C. Burri**. 2005. Effectiveness of a 10-day melarsoprol schedule for the treatment of late-stage human African trypanosomiasis: confirmation from a multinational study (Impamel II). The Journal of Infectious Diseases **191**:1922-1931.

21. **Van Nieuwenhove, S.** 1999. Present strategies in the treatment of human African trypanosomiasis, p. 253-281. *In* M. Dumas, B. Bouteille, and A. Buguet (eds.), Progress in human African trypanosomiasis, sleeping sickness. Springer, Paris.

22. **WHO**. 1998. Control and surveillance of African trypanosomiasis. WHO Technical Report Series **881**:1-113.

23. **Zhou, X.-H., N. A. Obuchowski, and D. K. McClish**. 2002. Statistical methods in diagnostic medicine, p. 1-464. John Wiley and Sons, New York.

393 Figure 1: Overview of study profile.



394 Table 1: Pre-treatment median values of the cerebrospinal fluid (CSF) white
395 blood cell (WBC) count, the intrathecal IgM fraction (IgM_{IF}), the CSF IgM
396 concentration, the CSF LATEX/IgM end titer, the CSF IL-10 concentration, the
397 CSF total protein concentration, and the serum IgM and IL-10 concentration in
398 cured and relapsed hemo-lymphatic stage patients. Differences were tested with
399 the Kruskal-Wallis test.

400

parameter	Median (interquartile range)		*p*
	Cured patients (n=46)	Relapsed patients (n=14)	
CSF WBC (cells/µl)	2 (1-3)	3 (1-4)	0.63
IgM_{IF} (%)	0 (0-0)[a]	50 (5-61)[b]	<0.0001
CSF IgM (mg/l)	0.82 (0.48-1.79)	3.33 (1.97-7.5)	0.0005
CSF LATEX/IgM (end titer)	0 (0-2)	2 (2-8)	0.0003
CSF IL-10 (pg/ml)	5.7 (1.9-7.4)	8.3 (5.2-14.7)	0.022
CSF total protein (mg/l)	344 (291-401)	318 (293-402)	0.88
Serum IgM (g/l)	4.53 (2.48-7.5)[a]	5.13 (2.76-8.5)[b]	0.59
Serum IL-10 (pg/ml)	90 (45-139)[a]	124 (93-136)[b]	0.14

401 [a] n=44; [b] n=13

402 Table 2: Number of cured and relapsed patients after suramin treatment in
403 function of pre-treatment test results, the *p* value (calculated by Fisher exact test)
404 and odds ratio (OR) with confidence interval (95% CI) for association of test
405 result with occurrence of relapse. CSF cerebrospinal fluid, IL interleukin

	Cured	Relapsed	*p*	OR (95% CI)
Intrathecal IgM synthesis (n=57)				
Absent	41	3		
Present (IgM_{IF}>0%)	3	10	0.0000015	46 (8.0-260)
CSF IgM concentration (n=60)				
<1.9 mg/l	35	3		
≥1.9 mg/l	11	11	0.0004	11.7 (2.7-50)
CSF LATEX/IgM end titer (n=60)				
<2	34	3		
≥2	12	11	0.001	10.4 (2.5-44)
<4	40	8		
≥4	6	6	0.024	5.0 (1.3-20)
<8	41	8		
≥8	5	6	0.014	6.2 (1.5-25)
CSF IL-10 (n=60)				
≤10 pg/ml	40	8		
>10 pg/ml	6	6	0.024	5.0 (1.3-20)

406