INVESTIGATIONS ON
THE ENCEPHALOPATHIC SYNDROME
DURING MELARSOPROL TREATMENT OF
HUMAN AFRICAN TRYPANOSOMIASIS

by
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During the thesis, the author worked in Lisbon and Basel, under the supervision of Professor Jorge Atouguia, from the Instituto de Higiene e Medicina Tropical and of Dr. Christian Burri, from the Swiss Tropical Institute.
To the sleeping sickness patients, who suffer and die,
their lament hardly heard
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When you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, your knowledge is of a meagre and unsatisfactory kind

Lord Kelvin, Professor of Natural Philosophy, Glasgow, 1846
Summary

Human African trypanosomiasis (HAT, sleeping sickness) has re-emerged in sub-Saharan Africa, placing an estimated 60 million people at risk of contracting the disease and eventually dying from it. HAT is a neglected disease. The limited available resources are preferentially directed towards disease control activities. The development of new drugs for HAT treatment is not commercially interesting from the pharmaceutical point of view. Given the characteristics of the rural African population affected, the return of investment in drug research would be insignificant.

With the introduction of melarsoprol into clinical practice in 1949, curative treatment for both forms (Rhodesiense and Gambiense) of late stage HAT became possible. However, a serious complication of melarsoprol administration soon became apparent, resulting in a fatal outcome in at least half of those affected. The phenomenon is presently designated the encephalopathic syndrome (ES). ES is responsible for half of the deaths occurring in late stage HAT and seriously jeopardizes melarsoprol otherwise generally high efficacy in late stage Rhodesiense and Gambiense HAT.

Despite more than half a century of extensive melarsoprol usage and considerable research efforts, critical aspects such as the definition, etiology and pathogenesis of ES are far from being satisfactorily studied or determined. Additionally, valid evidence concerning the risk factors for ES and the role of interventions for prevention and treatment of ES also remains to be acquired.

We designed and performed a dual methodological approach to the problem of ES in HAT.

The first is described in Chapter 2 and consists in a systematic review of the published and unpublished literature on ES, using the Cochrane Group methodology for systematic reviews.

The second is described in Chapter 3 and consists in a case control clinical study with prospective acquisition of clinical data, performed in 6 HAT treatment centres in Angola and in the Democratic Republic of Congo. The association between the HLA complex and ES was explored using state-of-the-art HLA molecular typing methods.

The combined approach obtained valid evidence on several important aspects of ES, but also showed that many other critical issues remain to be clarified.

A theoretical model for the etiology and pathogenesis of ES is proposed based on the combined findings of the dual approach. The proposed etiology of melarsoprol-related ES is an immune response occurring within the Central Nervous System (CNS), mainly mediated by T-cells, resulting in several degrees of severity of an autoimmune mistargeted attack to the brain capillary endothelium and brain parenchyma.
The proposed pathogenesis of ES involves a complex interaction between the parasite, the host and melarsoprol. Trypanosome and host related factors concur for the establishment of a favourable immunological environment in the CNS for the development of ES, which is subsequently triggered by melarsoprol.

A more precise definition of ES was obtained, which is useful in the correct diagnosis of the condition. The improved definition we obtained should also help in future studies on ES. We demonstrated that ES follows a main clinical pattern consisting in convulsions followed by coma. Incomplete presentations consisting in isolated convulsions or coma without convulsions are possible but these presentations are less frequent. Although described in the literature, ES consisting exclusively in mental manifestations of the psychotic type was not observed in the clinical study. Since we applied stringent definition criteria, we may speculate that ES consisting in this isolated type of manifestation is an observational bias or a much less frequent presentation of ES.

The combined search for risk factors showed that they are elusive. This is probably related to the multifactorial nature of ES and to the multiplicity of interventions used for its prevention, requiring large numbers of patients to confer adequate statistical power to the studies. However, factors such as concomitant infection and possibly a particular type of HAT-associated CNS damage appear to be significantly correlated with ES or with a greater risk of dying from ES.

Evidence on the role of the multiple drugs and interventions used for the prevention and therapy of ES, all empirically developed, is not conclusive. However, ineffective preventive interventions were detected. The therapy of a critically ill, convulsing or comatose ES patient has to be effective and the least possible toxic. Potential dangerous drug interventions and combination of interventions used in the management of ES patients are described. Several possibilities for the improvement of interventions aimed at the prevention and treatment of ES are proposed.

A significant statistical association between Class I haplotype C*14/B*15 and ES was found. Patients expressing this haplotype have a risk more than 6.5 times higher of developing ES. Additionally, three other haplotypes were also found to be possibly related to ES. This original finding-establishes the basis for future genetic studies that can lead to the clarification of the etiology and pathogenesis of ES.

Unfortunately, because of the lack of viable drug alternatives, melarsoprol will probably remain for many years the first-line treatment for both forms of late stage HAT, isolated or in combination therapy. Correctly designed and sufficiently powered clinical studies on ES, aimed at obtaining valid evidence on the etiology, diagnosis, risk factors and role of preventive and therapeutic interventions are an urgent necessity. The tool developed and tested in the
field during the clinical study allows a standardized prospective collection of
detailed clinical data. This tool may be used to gather reasonably
homogeneous data from different HAT treatment centres that may be pooled
to attain the statistical power needed to clarify the statistical trends described
in this work and to obtain valid and robust evidence on the risk factors and
on the role of interventions in ES.
Sumário

A tripanosomose humana Africana (THA, doença do sono) voltou a emergir na África sub-saariana, submetendo ao risco de infecção e subsequente morte um número de indivíduos estimado em 60 milhões. A THA é uma doença negligenciada. Os limitados recursos disponíveis são preferencialmente utilizados em actividades de controlo da doença. O desenvolvimento de novos fármacos para tratamento da THA não apresenta interesse comercial para a indústria farmacêutica, uma vez que, em função das características da população rural africana afectada, o retorno financeiro do investimento na pesquisa de novos fármacos seria irrisório.

Com a introdução do melarsoprol na prática clínica em 1949, tornou-se possível o tratamento curativo da THA em período neurológico nas suas duas formas (Rodesiense e Gambiense). No entanto, uma complicação séria do uso do melarsoprol tornou-se rapidamente evidente, conduzindo à morte pelo menos metade daqueles afectados. O fenómeno é actualmente designado por síndroma encefalopática (SE). A SE é responsável por metade das mortes que ocorrem no período neurológico da THA e diminui seriamente a eficácia geralmente alta do melarsoprol, tanto na THA Rodesiense quanto na Gambiense.

Apesar de mais de meio século de extenso uso do melarsoprol e de consideráveis esforços de investigação, aspectos críticos da SE, tais como a sua definição, etiologia e fisiopatogénese estão longe de ser satisfatoriamente estudados ou determinados. Além disso, permanece por adquirir evidência válida em relação aos factores de risco para a SE e ao papel das intervenções usadas na sua prevenção e terapêutica.

Concebemos e efectuámos uma aproximação metodológica dualística para o problema da SE.

A primeira, descrita no Capítulo 2 consistiu numa revisão sistemática da literatura publicada e não publicada sobre o assunto, utilizando a metodologia do grupo Cochrane para revisões sistemáticas.

A segunda, descrita no Capítulo 3 consistiu num estudo caso-controlo com aquisição prospectiva dos dados clínicos, realizado em 6 centros de tratamento de THA em Angola e na República Democrática do Congo. A possível associação entre o complexo HLA e a SE foi explorada utilizando métodos "state-of-the-art" para tipagem molecular do HLA.

A aproximação dualística permitiu obter evidência válida em relação a vários aspectos importantes da SE, mas mostrou igualmente que questões críticas permanecem por esclarecer.

Um modelo teórico para a etiologia e a fisiopatogénese da SE é proposto baseado nos achados combinados da aproximação dualística. A etiologia proposta para a SE consiste numa resposta imunológica mediada principalmente por linfócitos T, que ocorre no sistema nervoso central
(SNC), e que resulta em vários graus de severidade de um ataque autoimune erroneamente dirigido contra o endotélio capilar e parenquima cerebrais.

A fisiopatologia que propomos para a SE envolve uma interação complexa entre o parasita, o hospedeiro e o melarsoprol. Factores ligados quer ao tripanossoma quer ao hospedeiro contribuem para a existência de um ambiente imunológico no SNC favorável ao desenvolvimento da SE, que é subsequentemente despoletada pelo melarsoprol.

Uma definição mais precisa da SE foi obtida, útil no diagnóstico correcto da patologia. A melhor definição obtida pode ser igualmente útil em estudos futuros sobre a SE. Demonstrámos que a SE segue um padrão clínico principal que consiste em convulsões seguidas de coma. Apresentações clínicas incompletas, consistindo em convulsões isoladamente ou em coma sem convulsões são passíveis de ser observadas, mas este tipo de apresentações são menos frequentes. Ainda que descrita na literatura, a SE com manifestações exclusivamente mentais do tipo psicótico não foi observada no estudo clínico. Uma vez que utilizamos criterios de definição estritos, podemos especular que a SE com este tipo de manifestação isolada constitua um viés de observação ou que a sua frequência seja muito menor.

A busca combinada de factores de risco para a SE mostrou que estes são intangíveis. Isto poderá estar relacionado com a natureza multifatorial da SE e com a multiplicidade de intervenções utilizadas na sua prevenção, necessitando-se um grande número de doentes para que os estudos tenham potência estatística adequada. No entanto, factores tais como infecções concomitantes e possivelmente um tipo peculiar de dano do CNS associado à THA, surgem como estando associados à SE ou ao risco acrescido de morte por SE.

A evidência sobre o papel dos múltiplos fármacos e intervenções usadas na prevenção e tratamento da SE, todas empiricamente desenvolvidas, não é conclusiva. No entanto, intervenções ineficazes foram detectadas. O tratamento de um doente com SE em estado crítico, com convulsões ou em coma, deve ser eficaz e o menos tóxico possível. Descrevemos intervenções medicamentosas e combinações medicamentosas potencialmente perigosas usadas no tratamento da SE. Várias possibilidades de melhoria das intervenções destinadas à prevenção e tratamento da SE são propostas.

Uma associação estatisticamente significativa entre o haplótipo de Classe I C*14/B*15 e a SE foi encontrada. Portadores deste haplótipo tem um risco mais de seis vezes e meio maior de desenvolver SE. Foram encontrados mais três haplótipos que poderão adicionamente estar associados à SE. Este resultado original estabelece a base para estudos genéticos futuros que poderão levar à determinação da etiologia e patogênese da SE.
Infelizmente, em função da ausência de alternativas terapêuticas viáveis, o melarsoprol, isoladamente ou em terapêutica combinada, permanecerá provavelmente durante muitos anos o tratamento de primeira escolha para o período neurológico da THA nas suas duas formas. Estudos clínicos de concepção correcta e com potência estatística adequada, visando obter evidência válida sobre a etiologia, o diagnóstico, os factores de risco e o papel das intervenções preventivas e terapêuticas, são uma necessidade urgente. A ferramenta desenvolvida e testada no terreno durante o estudo clínico permite uma aquisição prospectiva padronizada de dados clínicos detalhados. Esta ferramenta pode ser usada para acumular e juntar dados razoavelmente homogéneos provenientes de diferentes centros de tratamento de THA, de forma a poder atingir a potência estatística necessária para o esclarecimento das tendências estatísticas observadas neste trabalho, assim como a obtenção de evidência válida e reproduzível sobre os factores de risco e ao papel das intervenções na SE.
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PART I - GENERAL INTRODUCTION

1 HUMAN AFRICAN Trypanosomiasis

1.1 GENERAL ASPECTS

Human African trypanosomiasis (HAT) or sleeping sickness (SS) is caused by the protozoan parasites *Trypanosoma brucei gambiense* (Gambian or West African form of the disease) and *Trypanosoma brucei rhodesiense* (Rhodesiense or East African form of the disease). Trypanosome infected blood sucking tsetse flies (*Glossina sp.*) transmit the disease to man. The occurrence of sleeping sickness is linked to the existence in the same geographic setting of the three essential elements that determine transmission: tsetse flies, trypanosomes and mammalian hosts, including man. Sleeping sickness is an exclusively African disease and its distribution is chiefly determined by the peculiar environmental and climatic requirements of the tsetse flies. Following inoculation, infection remains temporally limited to the haemolymphatic system during early stage disease but will eventually progress to central nervous system (CNS) invasion and late stage disease. The Rhodesiense form generally kills the infected patient in weeks or months while the Gambiense form takes a more chronic course, lasting for months or years. Severely neurologically and mentally incapacitated, the late stage patient becomes a burden for society before he eventually dies. It is in this extremely high morbidity and lethality that lays the principal final impact of HAT in terms of individual and public health, since an important percentage of infected patients will die unless effective treatment is instituted. African trypanosomiasis, without intervention, has the propensity to develop into epidemics, making it a major public health problem (WHO, 2001).

1.1.1 Historical background (*)

In early African oral tradition sleeping sickness receives names like “sleepiness” or “nut-disease”, corresponding to the identification of the late stage symptoms. Attributed names like “river “or “fishers” disease also
indicates that risky sites for transmission were correctly identified. Several traditional practices, including the excision of the posterior neck glands, were and probably still are performed by local healers to cure sleeping sickness.

However, the characteristics of human settlements in early African civilizations apparently did not favour the occurrence of epidemics. The scarcity of the population, the habit of clearing vegetation in large areas around villages and the existence of densely grown areas separating tribes and kingdoms helped to avoid the spread of the disease from one community to another. Dated from 1373, the report by historian Ibn Khaldun of the death of King Diata II, sultan of Mali, is considered the first known written record on sleeping sickness.

Initiated in the 15th century by the Portuguese and followed by the French and British, the colonial slave trade took millions of Africans overseas. Medical officers stationed in the Atlantic coast stations, ship doctors and slave traders were aware of sleeping sickness. As reported by Winterbottton in Sierra Leone (1803), swollen cervical lymph nodes were considered by slave traders as an ominous sign and an indicator that the slave should not by bought. Livingstone provides the first description of the harmful effects of tsetse flies in his “Missionary Travel and Researches in South Africa” (1857).

European explorers, missionaries, commercial agents, soldiers and colonial personnel invaded the interior of Africa in the late 19th century. Expansion required the occupation of unexplored environments and huge population displacements. The long established micro-ecosystems and traditional relationships were severely disrupted, causing the breakdown of the ancient “sanitary barriers”. In Central Africa for instance, the spread of sleeping sickness is shown to follow the occupation of the Congo River as far as the shores of Lake Victoria. The first histopathological description of HAT is published in 1899 by Mott. In the brains of two Congolese HAT patients Mott describes the perivascular mononuclear infiltration around brain vessels
and the typical large abnormal plasma cells, that latter became known as “morula cells of Mott”.

By decimating large numbers of individuals and rendering land non-exploitable, sleeping sickness and animal African trypanosomiasis became a serious nuisance for colonial powers. Scientific missions were organized between 1900 and 1915 by the Portuguese (Angola and São Tomé e Príncipe: Bettencourt, Kopke, de Rezende and Mendes, 1901-02), British (Uganda: Low, Christy, Castellani and Bruce, 1902-03) French (French Congo: Martin, Leboeuf and Roubeaud, 1906-08), German (German East Africa: Koch, Kleine and Beck, 1906-07) and Belgian (Congo: Dutton, Todd, and Christy, 1903-05) authorities to deal with the problem. Between 1901 and 1905, the etiology (Forde, Dutton, Castellani and Bruce), the transmission and the epidemiology of sleeping sickness (Christy, Brumpt and Bruce) were described. The first effective treatment in humans was performed in 1905 (Kopke and Koch) using the aromatic arsenical known as Atoxyl, synthesized by Béchamp in 1863 for treatment of anemia and skin diseases.

Massive campaigns were launched to gain control over sleeping sickness in sub-Saharan Africa. Different strategies were adopted depending on the geographical setting. The first eradication of HAT transmitting Glossina was obtained by the Portuguese in 1911 in the Príncipe Island (Guinea Gulf). In East Africa, vector control by means of tsetse trapping and DDT use was particularly successful. For the Gambiense form of HAT, Jamot (1879-1937) created an effective disease control system, consisting in systematic case detection and treatment by mobile teams covering the highest possible population at risk, aimed at eliminating the parasite reservoir. The system was entirely vertical, with specially trained personnel exclusively dedicated to HAT and an absolute autonomy in terms of technical, administrative and budgetary matters. Diagnosis of sleeping sickness was done by the direct examination of blood, lymph node aspirate and cerebrospinal fluid (CSF) for the presence of trypanosomes. Usually a threshold of 5 cell/mm$^3$ count in CSF was applied
for stage determination, sometimes associated with CSF total protein concentration determination.

Following the spectacular results obtained by Jamot in Cameroon (1926-1930), British, Belgian and Portuguese sleeping sickness services were also successfully organized according to his principles in Ghana, Nigeria, Congo, Angola and latter in Mozambique and Portuguese Guinea. An idea of the dimension of the complex operation of a sleeping sickness service is given by the reported 50'000 lumbar punctures performed in one single year (1946) in French West Africa. By the late sixties the overall percentage of new cases of *T. b. gambiense* disease had fallen to below 0.01%. However, a residual level of incidence persisted and eradication was never achieved.

The existence of effective drugs was critical for the success of the disease control strategy. Drug development proceeded empirically, mainly based on Paul Ehrlich’s (1854-1915) pioneering work with dyes and arsenicals started in 1899, when he was appointed director of the Royal Institute of Experimental Therapy in Frankfurt. In 40 years, *circa* 12’000 compounds were screened for potential clinical efficacy. Efficacious against first stage disease, suramin (for both forms of HAT) and pentamidine (only for Gambian disease) were introduced in 1920 and in 1939, respectively. The therapeutic choice for late stage disease remained limited to the rather inefficient Atoxyl-derived compound tryparsamide, introduced in 1919 by Jacobs and Heidelberger. Therapeutic failures with tryparsamide started however to be increasingly reported all over Africa.

It was only in 1949, with the introduction of the new arsenical drug melarsoprol, that a curative treatment for both forms of late stage HAT became available. Melarsoprol was obtained by Friedheim (1899-1989), a Swiss medical pathologist and microbiologist, by combining the organic arsenical melarsen oxide, which he developed, with the heavy metal chelator know as British Anti-Lewisite (BAL, dimercaprol), which was used during
World War II as an antidote for arsenical-based nerve gases. The compound obtained by “capping” melarsen oxide with BAI retained a high trypanocidal activity and toxicity was lowered to acceptable levels. Melarsoprol showed to be effective in *T. b. gambiense* first stage disease with one single application. Parasitological cure of late stage disease required at least 3 applications. Following extensive experimentation with several empirically designed administration schedules, melarsoprol became the most widely used drug for both forms of late stage HAT, bringing the hope of cure to previously condemned patients.

(*) The historical aspects described are based on: (Friedheim, 1949), (Dutertre, 1968), (Atouguia, 1998), (Ollivier and Legros, 2001), (WHO, 2001), (Legros, Ollivier et al., 2002), and (de Raadt, 2001).

1.1.2 Present situation

Since the disease had been brought to very low incidence levels in the late 60’s, the emerging independent African governments gave a low priority to HAT control activities, allowing the disease to return insidiously. Additionally, over the past four decades, constant war, overpopulation, migrations, inadequate handing-over of the responsibilities of government by colonizing powers, associated with the almost exclusively rural and multifocal distribution of the disease, have resulted in the disruption of national control programs and have contributed to the re-emergence of HAT (Burri, 2001).

It is estimated that 60 million people are at risk of contracting HAT in 36 counties in sub-Saharan Africa, and that at least 45'000 to 50'000 individuals become infected each year. Although the precise number of infected individuals is not easy to obtain, it is thought to be in the 300'000 to 500'000 range (WHO, 2001). Only 4–10% of the population at risk is under surveillance or has access to adequate specialized medical services. The number of deaths directly caused by human African trypanosomiasis is estimated at 66 000 per year, so the total number of patients is self limiting
and decreasing. In some regions, this number has become similar or superior to that of AIDS-associated fatalities. The main burden in terms of human disease is given by *T. b. gambiense* infection (Cattand, 2001). After malaria, leishmaniasis and helminthic diseases, Gambiense HAT is the fourth parasitic disease in terms of loss of healthy life years by premature mortality and disability (Disability Adjusted Life Years, DALY) in tropical Africa (http://www.who.int/tdr/diseases/default.htm).

The range of tsetse infested areas goes from latitudes 14º north to 29º south of the equator, roughly from the Sahel to the Kalahari Desert. This distribution makes 10 million square kilometres of land non-exploitable for animal husbandry, hunting, fishing or crop-raising activities. Sleeping sickness has a multifocal distribution that reflects the presence of tsetse flies, trypanosomes and humans in the same geographical and temporal setting. The two forms of HAT are geographically separated roughly along the line of the Great Rift Valley. Rhodesiense trypanosomiasis is observed to the east of this line and Gambiense to the west. Rhodesiense trypanosomiasis is a zoonosis, with a wide variety of wild and domestic mammalian animal species acting as the actual or potential parasite reservoir (Hutchinson, Fevre et al., 2003). In Gambiense disease man is the main parasite reservoir, but the importance of animal reservoirs is presently under discussion (Jamonneau, Barnabe et al., 2003). Presently around 250 active foci are recognised. Countries are classified by the WHO according to their transmission status (Figure 1).
The present situation, numerically similar to the one encountered in the beginning of the 20th century is by many specialists considered in fact as being worse and calamitous, since the at risk population is so poorly covered by screening activities (Figure 2). In opposition to the relative socio-political stability prevalent during colonial times, the present situation is characterized by social, political and military turmoil. In the three countries that concentrate 90% of Gambiense HAT cases (Angola, the Democratic Republic of Congo and Sudan) the infected and at risk population is almost exclusively rural and has very limited access to health care. In Angola, where one fifth (2.5 million) of the population is estimated to be at risk for infection, the situation is particularly dramatic. Due to the collapse of health care infrastructures, insecurity and landmines, only 6% have access to adequate HAT surveillance and treatment (Stanghellini and Josenando, 2001). In the Democratic Republic of Congo, the annual case detection rate in 1994 was twice that of
1930. In 1998, only a small portion (12%) of the population at risk was estimated to be under effective surveillance (Van Nieuwenhove, 2001). In Sudan the prevalence now exceeds 5%, with an estimated total of 1-2 million individuals living in areas at risk (Moore, 2001). Urban transmission of sleeping sickness has been observed in Kinshasa and may be an emerging problem (Louis, Bilenge et al., 2003).

One of the consequences of the national control programs breakdown is that only 10-15% of the infected population is diagnosed and treated (Cattand, 2001). This will lead to a large number of individuals remaining infective and amplifying the epidemic. Another important consequence is that a significant percentage of patients will tend to be in late stage disease when finally diagnosed.

Recent reports of Rhodesiense HAT in travellers have drawn attention to the risk of acquiring the infection in game reserves in Eastern Africa (Moore, Edwards et al., 2002), (Ripamonti, Massari et al., 2002). After a serious epidemic in the 1980’s in southeast Uganda, *T. b. rhodesiense* disease is now quiescent in most East and southern Africa countries (Welburn and Odiit, 2002). However, a new *T.b. rhodesiense* focus was identified in 1998 in the Soroti district, Uganda (Hutchinson, Fevre et al., 2003). This country is the only one where foci of both forms of HAT co-exist.

Disease control activities, either in tsetse fly control or in the active and passive search for infected individuals and in the delivery of efficacious treatment, are heavily jeopardized because of insufficient interest from central authorities, lack of funds, infra-structural and logistic difficulties, moving populations, land mines and ongoing military activities (Burri, 2001). Today, sleeping sickness is considered as a typical example of a neglected disease (Jannin, Simarro et al., 2003).
In the present African context of an extreme lack of human and logistic resources, new approaches and optimized old ones using new tools have been developed to help in HAT control activities, mostly under the incentive from World Health Organization / Tropical Disease Research (WHO/TDR) (Louis, Simarro et al., 2002).

HAT distribution can now be more precisely mapped thanks to geographic information systems (GIS), allowing a more precise targeting for screening activities, the surveillance of treatment needs and resistance emergence, as
well as vector control activities (Cattand, 2001). This is particularly critical
given the lack of funds and the difficulties in access to remote foci. Linking
GIS data with mathematical models for HAT transmission may bring further
knowledge on the needs for vector and mammal host disease control in the
diverse foci (Gouteux and Artzrouni, 1996), (Muller, Grebaut et al., 2004).

Studies on the biology of trypanosomes require access to stable parasite
genotypes. This was made possible by the introduction of cryopreservation
techniques (Cunningham, Lumsden et al., 1963), (Lumsden, Cunningham et
al., 1963). Trypanosomes can presently be identified with increased precision
by molecular biology tools and isoenzyme analysis. Similarities and differences
between T.b. gambiense, T.b. rhodesiense and animal trypanosomes are becoming
clearer (Gibson, 2003). Sequencing of the African trypanosome genome is
under way at The Institute for Genomic Research and at the Sanger Centre.
Thanks to these advancements the complex relationships between
trypanosomes and mammal hosts are better understood (Welburn and Odlit,
2002), (Agbo, Clausen et al., 2003), (Jamonneau, Barnabe et al., 2003). In
humans, disease severity is associated with different parasite genotypes but
also probably with host-related factors such as ageing, immunity and genetic
background (Garcia, Jamonneau et al., 2002), (Jamonneau, Garcia et al.,
2002).

Tsetse eradication was accomplished on the island of Zanzibar (Tanzania) in
1997, using a combination of conventional methods and the sterile insect
technique (SIT) (Vreysen, Saleh et al., 2000). Created in 2000, The Pan
African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC)
initiative seeks the eradication of tsetse flies in Africa by means of area-wide
tsetse control strategies using this combination of methods (see

The identification of parasite biochemical and molecular targets, the
development of proteomics, high out-put compound synthesis and
optimized screening methods for compound bioactivity, allow drug search and design to presently proceed in a rational way. However, from the pharmaceutical point of view, sleeping sickness is not a commercially viable disease since the financial return of the high costs of research and drug development for a compound exclusively aimed at HAT treatment is insignificant (Atouguia and Costa, 1999).

An innovative approach to tackle the HAT problem consists in establishing joint public and private initiatives that bring to close collaboration pharmaceutical companies, academia and international agencies, to support the development of state-of-the-art diagnostic and therapeutic tools.

In 2001, the collaboration between the Drugs Working Group of the WHO Sleeping sickness treatment and resistance Network, pharmaceutical companies and the non-governmental organization “Médecins sans Frontières” was able to secure the endangered production and distribution of the current existing drugs for HAT treatment for a period of five years.

A promising new oral treatment (the diamidine derivative DB 289) for the treatment of early stage Gambiense HAT is under clinical investigation by a Consortium led by the University of North Carolina and sponsored by the Bill and Melinda Gates Foundation. A phase IIA clinical trial (proof of concept) was completed in Angola and in the Democratic Republic of Congo (DRC) (Legros, Ollivier et al., 2002) and the drug is presently under extended trial (phase IIB) in the DRC (Burri, personal communication). However, even if clinical trials are successful, DB 289 will probably not reach the market for at least 5 years. There are presently no other new drugs under clinical development. Megazol (a nitrothiazole derivative) is highly active against several species of trypanosomes, including T. cruzi and T.b. brucei that has been in pre-clinical development for several years (Marie Daragon, Rouillard et al., 1994), (Barrett, Fairlamb et al., 2000). Megazol is orally bio-available and effectively crosses the BBB in the monkey model (Enanga, Ndong et al.,
2000). However, in vivo megazol genotoxic potential has recently been confirmed to be very high. Passage into clinical development is presently not recommended until less genotoxic megazol analogues are found (Nesslany, Brugier et al., 2004).

Awareness about sleeping sickness and animal African trypanosomiasis is rising (http://www.who.int/tdr/diseases/tryp/direction.htm). Continued concerted efforts coming from research scientists, field workers, development agencies and companies are needed to technically achieve control over African trypanosomiasis. Furthermore, politicians must be willing to see the parallel between civil conflict and social instability and HAT epidemics. The example of the substantial economical impact of the successful American trypanosomiasis (Chagas disease) control programs may also possibly help convince politicians of the advantages of establishing control over African trypanosomiasis. For every dollar invested in Chagas disease control in Brazil (in a 6 million square kilometres area), the return is estimated in more than 7 USD, far out weighting the return of programmes against malaria, leishmaniasis, dengue and AIDS (Vinhaes and Schofield, 2003). Considering the potential for regional development and poverty reduction, the impact of African trypanosomiasis control might be even greater and represent good value for the money invested. Furthermore, since the case fatality rate in untreated patients is 100%, the number of DALYs averted per infection cured or prevented are very high: control of sleeping sickness in endemic areas is highly cost-effective, falling well below the accepted threshold value for money of US$25 per DALY averted (Shaw and Cattand, 2001).

1.2 The parasite

African trypanosomes are unicellular eukaryotes that infect animals and humans. They are classified in the sub-kingdom Protozoa, the phylum Sarcomastigophora, the order Kinetoplastidae, the family Trypanosomatidae and the genus Trypanosoma. The development of the infective metacyclic form of the parasite may take place whether in the intestine (Section Stercoraria) or
in the salivarian glands (Section Salivaria) of the vector. The sole human pathogenic Stercoraria member is *Trypanosoma cruzi*, the agent of American trypanosomiasis (Chagas disease). The Salivaria are further sub-divided into the subgenera Tejeraia, Duttonella, Nannomonas, and Pycnomenas, chiefly infecting cattle and small ruminants. A fourth subgenus, Trypanozooon, comprises 3 species: *T. equiperdum*, *T. evansi* and *T. brucei*. The first two species are of veterinary importance, causing disease in horses (“dourine”) camels, cattle and buffaloes (“surra”). The subspecies *T. brucei brucei* is the agent of “nagana” in cattle and small ruminants. *T. b. brucei* is lysed by human serum and generally considered not infective for man. Only two subspecies of *Trypanosoma brucei*, i.e. *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are presently recognized as causing disease in humans (WHO, 1986).

This classification is nowadays the subject of discussion under the light of mathematical methods applied to progress obtained by biochemical and molecular characterization studies, establishing relationships between animal and human trypanosome species (Godfrey, BAker et al., 1990), (Stevens and Godfrey, 1992), (Gibson, 2003). The critical issue of precisely determining which subspecies and strains are pathogenic or potentially pathogenic to man remains one of the most important questions to be answered. Differentiation between *T.b. rhodesiense* and *T.b. gambiense* can presently be established by several molecular methods (Gibson, 2001). However, the same methods failed to distinguish human *T.b. rhodesiense* from animal *T.b. brucei* species. The situation changed when a gene conferring resistance to the trypanolytic effect of human serum (human serum resistance associated gene, SRA) was isolated and characterized from a Ugandan strain of *T.b. rhodesiense* (de Greef, Imberiets et al., 1989), (de Greef and Hamers, 1994). SRA is absent in *T.b. brucei* non-human infective species and is sufficient to confer resistance on *T.b. brucei* by transfection (Xong, Vanhamme et al., 1998). It was subsequently demonstrated that SRA is ubiquitous and conserved throughout East Africa (Gibson, Backhouse et al., 2002). The issue of differentiation between
pathogenic and non-pathogenic parasites is further complicated by the findings that African trypanosomes can undergo genetic exchange in the tsetse fly (Sternberg and Tait, 1990), (Bingle, Eastlake et al., 2001) and that patients can harbour multiple T. b. gambiense genotypes (Truc, Ravel et al., 2002). Additional work is required before we can fully understand the complex relationship between trypanosomes, humans and animals.

The ultra-structure of T. brucei subspecies is well described. Morphology does not allow differentiation between T. b. brucei, T. b. gambiense and T. b. rhodesiense. Trypanosomes exhibit specialized sub-cellular structures that perform unique vital functions (reviewed by de Souza, 2002).

Energy generation is performed in conjunction by a number of glycolytic enzymes, normally located in the cytosol in other eukaryotic organisms, but sequestered in glycosomes and in the single mitochondrion in trypanosomes. The biochemical pathways for trypanosome metabolism are well studied, allowing the targeting of several trypanosome specific enzymatic reactions for therapy (Oppendoes and Michels, 2000), (Hannaert, Bringaud et al., 2003) (Figure 3).
The parasite surface membrane is coated by a dense layer of glycoprotein molecules (Variable Surface Glycoprotein, VSG) which are linked to the surface membrane by a Glycosyl-Phosphatidil-Inositol anchor (GPI). Ten million copies of a single species of VSG cover the trypanosome surface at any one time. VSG coating renders the parasite virtually impermeable to the diffusion of macromolecules (Pays, 1999). The “Achilles heel” in the trypanosomal surface appears to be the flagellar pocket, a membrane invagination intimately associated with the kinetoplast, where endo- and exocytosis takes place (Hoare, 1972), (de Souza, 2002). Trypanosomes undergo multiple and complex structural and metabolic changes during their life cycle. The critical steps are indicated in Figure 4.
The habitat of trypanosomes varies from the anaerobic conditions in the midgut of the tsetse to the aerobic, glucose rich conditions in the bloodstream and CSF of the human host. Trypanosomes can be found in man as an extracellular spindle-shaped, curved, flat, flagellated, highly motile parasite (figure 5). The existence of intracellular trypanosomes has been suggested but not fully documented. T. b. brucei may be observed in intercellular space in the interstitial tissue of the heart muscle, the pancreas and the choroid plexus (reviewed by Lonsdale-Eccles and Grab, 2002). Two forms are seen in blood and in vitro culture: one slender and one stumpy. The short stumpy form does not divide. When ingested by glossecta it efficiently adapts to the loss of glucose as the source of energy (using instead proline) and undergoes morphological changes that lead to proliferative procyclic trypomastigotes in
the insect midgut and subsequently to infective metacyclic forms in the salivary glands. Slender forms, which do not exist in the insect, are metabolically very active and constantly multiply by binary fission, with a population doubling time of 6 hours. Trypanosomes constantly change the VSGs on their surface. The resulting antigenic composition of the coat also changes periodically, producing Variant Antigenic Types (VAT) trypanosomal populations. This is the basis for the immune evasion in trypanosomes. The gene repertoire for VSG (1% of the total trypanosome genome, i.e. 1’000 genes) is vastly sufficient to allow survival in the host for long periods (years in T. gambiense disease). Additional knowledge on the exact mechanisms for the transcriptional control of VSG genes is needed and could provide new targets for therapy (McCulloch, 2004), (Matthews, Ellis et al., 2004). The complexity of the trypanosome life-cycle and this sophisticated immune evasion mechanism makes the development of an efficacious vaccine improbable in the near future (Legros, Ollivier et al., 2002).

![Figure 5: Blood stream form of human trypanosome (Source: Public Health Image Library)](image)
1.3 The Disease

1.3.1 Pathogenesis and pathology

Following inoculation of metacyclic infective forms into the skin, trypanosomes enter the blood stream and lymph vessels, producing parasitemia. They subsequently invade the Central Nervous System (CNS).

To be able to survive in the host’s extra-cellular milieu, trypanosomes continually change their outer coat antigenic composition. The immune system, mainly by means of a B-lymphocyte mediated humoral response, is constantly targeting and destroying trypanosomes. Remission periods correspond to the elimination of more than 90% of the population of trypanosomes expressing a given VAT by antibodies (mainly of the IgM class), combined with complement-mediated lysis, synthesis of cytostatic/cytotoxic molecules, opsonization-facilitated phagocytosis and antibody dependent cell-mediated cytotoxicity. Trypanosomes expressing different VSGs are unaffected by VAT-specific antibodies and as they escape destruction and continue to divide a new wave of parasitemia develops (reviewed by McCulloch, 2004). Release of pro-inflammatory mediators contributes to constitutional signs and symptoms such as fever and malaise, to an elevated erythrocyte sedimentation rate and to activation of the kallikrein-kinin and complement pathway systems. A marked reactivity of the lymphoid tissue with predominance of plasma cells is seen. Polyclonal B lymphocyte activation results in intense immunoglobulin synthesis. Ultimately ineffective antibodies accumulate in the host’s serum (Greenwood and Whittle, 1980). Autoimmune phenomena such as auto-antibodies and immune complexes develop, and a progressive depletion of lymphocytes and plasma cells is established (Vincendeau, Jaubertau et al., 1999). These cells are largely replaced by INF-γ activated macrophages. Stimulation of CD8⁺ T-cells to produce INF-γ is mediated by a protein factor released by T. brucei, the \( T. \) \( \text{brucei} \)-derived-lymphocyte-triggering-factor (TLTF) (Bentivoglio, Grassi et al., 1994).
A profound dysregulation in the cytokine network is observed, with overproduction of TNF-α, INF-γ, IL-1β, IL-6, IL-10 and PGE (Askonas, 1984), (Rhind, Sabiston et al., 1997), (Lejon, Lardon et al., 2002). Induction of NO synthetase results in high levels of NO (Vincendeau, 1992), (MacLean, Odiit et al., 2001). A possible mechanism for the suppressive activity of macrophages on immune effector cells includes faulty processing of trypanosome antigens and their inadequate presentation with MHC class II products (Pentreath, 1991). The final result (mainly measured in the animal model and in vitro studies) is suppression of the host immune response.

The pathogenesis of the complex progressive neuropathology of chronic sleeping sickness is not well understood. How the trypanosomes survive the apparently hostile environment provided by the unique characteristics of the nervous system parenchyma and the special immune properties of nervous tissues is unclear. A considerable amount of experimental data has been obtained on the pathogenesis of late stage HAT on animal models, including host and parasite gene knockouts (reviewed by Kennedy, 2004). Only recently have the complex changes in cytokine and immune cell network in the CNS and blood of human subjects, started to be studied thanks to sophisticated analyses of the serum and CSF (Lejon, Lardon et al., 2002).

At the CNS level the disease is characterized by a diffuse multifocal meningoencephalitis. Late stage CNS damage is widespread, but is particularly obvious in the white matter of the brain. Trypanosomes may be found in scarce quantity in CNS parenchyma, mainly in the weakest points of the blood brain barrier (BBB) (choroid plexus, circumventricular organs) (reviewed by Chimelli and Scaravilli, 1997). Choroid plexus damage, meningitis, astrocyte activation, perivascular cuffing with lymphocyte activation and microglial and astrocyte hyperplasia are observed. The parasite is able to enter the BBB without disrupting it possibly by transient leaks in an immunologically damaged barrier (Mulenga, Robertson et al., 2000). The pathogenesis of CNS damage and dysfunction results from a highly complex
interaction between parasite and host-derived factors (reviewed by Kennedy, 2004) (Figure 6).

1.3.2 Clinical manifestations

HAT must be seen as a continuum. The separation between early and late stage disease is artificial and reflects our ignorance on how and when the CNS is invaded and brain damage established. For surrogate markers of brain damage we rely on the presence of trypanosome in CSF, on CSF cell count and on CSF biochemistry. However, a patient with trypanosomes in CSF (and otherwise normal CSF) may not necessarily be on late stage as he may be cured by early stage drugs. Conversely, the absence of trypanosomes in CSF
does not necessarily rule out late stage as they may be demonstrated by more sensitive methods (Van Nieuwenhove, 1999). Neurological symptoms and signs may be present in patients staged both in early and late stage disease. They are generally considered as an indication that disease has progressed to the CNS and the patient receives CNS-active drug treatment.

The clinical symptoms and signs of HAT have been extensively reviewed (Cruz Ferreira and Lehman de Almeida, 1950), (Duggan and Hutchinson, 1966), (Apted, 1970), (Edan, 1979), (Boa, Traore et al., 1988), (Nkanga, Kazadi et al., 1988), (Molyneux, Pentreath et al., 1996), (Dumas and Bisser, 1999), (Atouguia and Kennedy, 2000).

1.3.2.1 Early stage
A chancre develops in the inoculation site within 5 to 15 days after the infective tsetse bite (the trypanosomal chancre, equivalent to the Romaña sign observed in Chagas disease). It consists in a cutaneous “furuncle without a head” that does not fluctuate and heals after 2 or 3 weeks by leaving a desquamative and discoloured scar. Satellite lymph node enlargement may exist. The chancre is infrequently diagnosed in endemic zones, either because cutaneous lesions are easily dismissed by rural patients or possibly because of previous exposure to tsetse bytes and non-pathogenic trypanosomes. The chancre is more frequent in Rhodesiense disease.

Blood, lymph node and tissue invasion may be detected within 1 to 3 weeks after inoculation. The onset of the disease is acute and quick in Rhodesian trypanosomiasis, but more gradual and slow in the West African form of the disease.

Constitutional, irregular, unspecific and inconsistent symptoms and signs such as fever, malaise, headache, fatigue, dizziness, generalized lymphadenopathy, arthralgia, pruritus and weight loss develop, lasting for one to seven days. The correct diagnosis is frequently missed and the patient is usually treated with antimalarials and/or antibiotics. After this the initial episode HAT takes an
intermittent course, with asymptomatic periods interrupted by episodes of fever that become shorter and shorter as disease progresses.

Lymphadenopathy is very common. Enlarged posterior cervical lymph nodes may become visible, in particular in Gambian disease (Winterbottom’s sign). Enlarged lymph nodes do not suppurate, they rather become fibrotic. Other manifestations of the involvement of the phagocytic-monomonuclear system include spleen and liver enlargement. These are neither prominent nor very helpful in diagnosis since they are very common in other tropical diseases.

A cutaneous rash may be present that often fades and reappears over a period of weeks. In its typical form, which is more easily observed in white skin, this rash is considered very specific of sleeping sickness. Pruritus in the absence of scabies or filarial infection is considered by some authors a strong evidence of HAT. Transient painless swelling of the face and in particular of the eyelids is recorded.

Other manifestations in the early phase of the disease are irritability, insomnia, personality change, loss of ability to concentrate, and somnolence. Endocrine dysfunctions include sterility, menstrual disorders, abortion, loss of secondary sexual characters, loss of libido and impotence.

Cardiac problems are more common in the Rhodesian form of the disease. Cardiac involvement may include tachycardia, lower intensity of cardiac murmurs, conduction defects, myocarditis (sometimes with congestive heart failure), and pericarditis or pancyarditis. In the foudroyant form frequently observed in Rhodesiense disease, cardiac involvement can lead to death, thus precluding the development of the typical manifestations of CNS involvement.
1.3.2.2 Late stage

The second or late stage of the disease is defined by CNS involvement. Some of the affected areas are those involved in the control of sleep. This phase is associated with severe modifications in the sleep-wake circadian rhythm that give the disease its name.

Neurological involvement is gradual, insidious and progressive, starting weeks or a few months after infection with *T. b. rhodesiense*. In Gambian trypanosomiasis, a period of one or more years may pass before CNS manifestations are severe enough to make the individual seek medical attention.

The clinical picture usually consists of a complex combination of mental, sleep and motor disturbances, neuro-endocrine dysfunction, and frontal lobe, vestibular, cerebellar, pyramidal and meningeal syndromes. Signs and symptoms depend on the intensity and topographic distribution of the CNS damage, and as for the clinical manifestations during early stage, they are not characteristic.

Mental disturbances are often subtle. Changes in personality and behavior are difficult to evaluate and may cause hospitalization of the patient in a psychiatric ward. Major presenting symptoms include an increasing indifference and lassitude, irritability, anxiety, agitation and maniac episodes sometimes with euphoria, uncontrolled sexual and suicidal impulses, and psychotic-like crisis with violent mood, delirium and hallucinations.

Motor system manifestations consist basically in tremors of distal predominance, muscle fasciculation, abnormal involuntary movements and increased muscular tonicity. Speech becomes slurred and slow, and ataxia leads to walking difficulties. Paralysis of one or more muscular groups is possible, affecting more frequently the lower limbs. Deep hyperaesthesia (Kérandel’s sign or “signe de la clé”) is sometimes observed. Abnormal reflexes such as the archaic peri-oral and cheiro-oral reflexes are the most
frequently seen. They are not pathognomonic of sleeping sickness but are considered as one of the first signs of late stage disease.

The most impressive characteristic of HAT sleep disturbance is the profound disruption of the circadian rhythm of the sleep-wake cycle and in the appearance of sleep episodes abnormally beginning with rapid eye movement periods (sleep onset rapid eye movement periods, SOREMP). This type of sleep disturbance is considered most typical of HAT. It was previously tough that these changes resulted in an inversion of the circadian rhythm, leading to daytime somnolence and nocturnal insomnia, but polysomnographic studies have demonstrated that the sleep-wake structure is rather anarchic (Buguet, Bourdon et al., 2002). Narcoleptic crisis can overtake the patient anywhere at any time, while walking or performing simple tasks such as eating.

1.3.2.3 Final stage
If left untreated the neurological manifestations of sleeping sickness progress to a final stage that includes epileptiform seizures, incontinence, intolerable generalized pruritus and a profound indifference towards the environment. Advanced neurological deterioration makes the patient lie immobile on the floor or hospital bed, rarely speaking, and never taking food spontaneously. The patient is usually severely debilitated and malnourished. Coma followed by death frequently results from a greater susceptibility and vulnerability towards bacterial infection.

1.3.3 Concomitant diseases
Due to their epidemiological distribution, malaria, filariasis and intestinal helminthic infections are frequently associated with HAT. The concurrent existence of other diseases such as coxsackie B infection, measles, pneumonia, tropical myositis, tuberculosis, leprosy, and bacterial meningitis has been documented and is associated with aggravation of the trypanosomal infection or with an increased incidence of treatment complications (reviewed by Atouguia and Kennedy, 2000).
The influence of HIV infection on the outcome of HAT is unknown. No significant epidemiological association was detected between HIV and HAT in Ivory Coast, Uganda or Central Africa, but further studies are needed to determine how the two infections influence each other (Noireau, Brun-Vezinet et al., 1987), (Meda, Doua et al., 1995). Deleterious interactions may be foreseen between trypanosomes and HIV in both directions (Harms and Feldmeier, 2002).

1.3.4 Diagnosis

The clinical diagnosis of HAT is hampered by the non-specificity of the clinical manifestations. Since no pathognomonic signs or symptoms exist, HAT may be suspected in face of the adequate epidemiological and clinical context but definitive diagnosis requires laboratory confirmation.

A fast and simple screening test detecting antibodies (exclusively for Gambiense infection) became available in 1978 (Magnus, Vervoort et al., 1978). This now widely used card agglutination test (CATT) helped improve diagnosis, follow-up and the performance of mobile teams in Gambiense endemic areas. Additional serological techniques (Immunofluorescence, ELISA) exist but they are seldom used in the field.

Parasite direct demonstration, generally in blood, lymph node aspirate and CSF is still mandatory for definitive diagnosis and to avoid unnecessary therapeutic risks with toxic drugs. Parasite identification may be difficult in Gambian disease due to the sometimes low parasite load. In Rhodesiense HAT the usually high and frequently constant parasitemia facilitates the diagnosis.

Concentration techniques such as the mini-Anion Exchange Centrifugation Technique (m-AECT) (Lumsden, Kimber et al., 1979) the capillary tube centrifugation (CTC) technique (Bennet, 1962), the Quantitative Buffy Coat (QBC) technique (Bailey and Smith, 1992) and the double centrifugation technique for CSF (Cattand, Miezan et al., 1988) improve the sensitivity of the
direct detection methods. They require more sophisticated equipment and personnel. *In vivo* cultivation of trypanosomes, the most sensitive method for diagnosis is too expensive and too complex to be applicable in the field. Increased specificity and sensitivity in diagnosis is presently obtained with DNA detection methods. These methods are however, expensive, not easily adaptable to field conditions and not yet validated (reviewed by Jamonneau, Solano et al., 2001) and (Gibson, 2002). Decisional algorithms are being developed to adequate and optimize the use of the diverse diagnostic methods available in the different epidemiological settings (Louis, Simarro et al., 2002).

1.3.4.1 *Stage determination*

Precise biological markers for determination of the neurological stage of disease are lacking. According to current WHO recommendations for stage determination the CSF has to be examined for the presence of trypanosomes, white blood cell (WBC) count and total protein concentration. If any of these parameters is abnormal (i.e more than 5 WBC/mm³ or a protein concentration above the cut-off of any of the existing methods) the patient is classified in late stage disease and treated accordingly. However, the threshold of normality for WBC count and protein concentration is arbitrarily fixed and is not necessarily entirely reflecting the trypanosome-induced pathogenic process within the CNS (reviewed by Lejon, Legros et al., 2001).

Alternative markers for late stage HAT have been proposed, consisting mainly in trypanosome specific antibody detection, trypanosomal DNA detection and IgM detection and quantification, all in the CSF. Recently, a simple, robust and thermostable latex card agglutination testkit for field detection of IgM in blood and CSF was developed (Lejon, Buscher et al., 1998) and successfully tested (Lejon and Buscher, 2002). Detection and dosage of the intrathecal synthesis of IgM has been confirmed as a sensitive method for stage determination. Field experience with the latex-IgM test shows that patients with 6 to 20 cells in CSF (the so-called intermediate stage
of HAT) can either by in early or late stage disease (Lejon, Reiber et al., 2003). Stage determination still requires lumbar puncture and remains a critical issue for the choice of the drug therapy of HAT.

1.3.4.2 Differential diagnosis

Many conditions can mimic both stages of HAT, especially in the tropics. It must be born in mind that more than one condition may also co-exist in the same patient. On the other, diagnosis of late stage HAT may be elusive outside of the African context, requiring a high suspicion index. The principal conditions that should be differentiated from HAT are shown in table 1.

<table>
<thead>
<tr>
<th>Acute conditions</th>
<th>Sub-acute or chronic conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>Tuberculosis (including CNS disease)</td>
</tr>
<tr>
<td>Typhoid and paratyphoid fever</td>
<td>Filarial infection</td>
</tr>
<tr>
<td>Infectious mononucleosis</td>
<td>Syphilis</td>
</tr>
<tr>
<td>Relapsing fever</td>
<td>Leprosy</td>
</tr>
<tr>
<td>Brucelosis</td>
<td>AIDS / HIV infection</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>Visceral leishmaniasis</td>
</tr>
<tr>
<td>Chagas disease</td>
<td>Chagas disease</td>
</tr>
<tr>
<td>Erysipelas, furunculosis</td>
<td>African histoplasmosia</td>
</tr>
<tr>
<td></td>
<td>CNS cryptococciosis</td>
</tr>
<tr>
<td></td>
<td>Lymphomas</td>
</tr>
<tr>
<td></td>
<td>Mental conditions</td>
</tr>
<tr>
<td></td>
<td>Cerebral tumors</td>
</tr>
</tbody>
</table>

Table 1: Differential diagnosis of HAT.

1.4 Treatment

1.4.1 Introduction

Few additional therapeutic options for HAT have been added to the ones introduced more than 50 years ago. Penetration into the CNS (i.e. the ability to cross the blood-brain barrier) divides drugs in two categories: those indicated for first stage HAT and those for late stage. The issue is further complicated by the different drug sensitivities exhibited by *T.b gambiense* and *T.b rhodesiense*. (Table 2). As a consequence of the currently limited drug
development activities, treatment will rely for a long time on presently existing drugs. While efforts are pursued to obtain the ideal cheap oral drug efficacious against both stages of both forms of HAT, the optimization in patient management with current drugs through improvement of the efficacy and reduction of serious drug reactions rate with is a priority (Van Nieuwenhove, 1999).

<table>
<thead>
<tr>
<th>Early stage</th>
<th>Late stage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Suramin:</strong></td>
<td><strong>Melarsoprol:</strong></td>
</tr>
<tr>
<td>both subspecies</td>
<td>both subspecies</td>
</tr>
<tr>
<td><strong>Pentamidine:</strong></td>
<td><strong>Eflornithine:</strong></td>
</tr>
<tr>
<td><em>T.b.gambiense</em></td>
<td>mainly <em>T.b.gambiense</em></td>
</tr>
<tr>
<td><strong>Diminazene aceturate:</strong></td>
<td><strong>Nifurtimox:</strong></td>
</tr>
<tr>
<td>both subspecies</td>
<td>mainly <em>T.b.gambiense</em></td>
</tr>
</tbody>
</table>

Table 2: Drug efficacy in human African trypanosomiasis: relation to disease stage and trypanosome subspecies.

1.4.2 Chemotherapy of early stage

1.4.2.1 Pentamidine

Pentamidine was introduced for treatment of HAT in 1940. Only one salt of this diamidine is available, pentamidine isethionate (Pentacarinat®), mesylate (Lomidine®) being no longer produced. The mechanisms of trypanocidal action are not well known but are related to trypanosome polyamine synthesis and respiratory activity depression. Administration is intramuscular or by slow intravenous infusion. Four mg/kg are given daily for a maximum of 10 doses and a minimum of 7 doses. Pentamidine has a low penetration into the CNS and a cumulative effect is observed after repeated administration. Hypotension, syncope, tachycardia, vomiting and shock may be seen immediately after injection. Local toxicity with necrosis and cold abscess formation at the injection site is common. Systemic toxicity includes nephrotoxicity, and transient hypo or hyperglycemia, and diabetes.
Pentamidine is mainly active against *T. b. gambiense* infections (reviewed by Nok, 2003) and Fairlamb, 2003).

1.4.2.2 *Suramin*

Suramin (Germin™) is a polysulphonated naphthylurea that was introduced for HAT treatment in 1920. Suramin trypanocidal action may be related to an opsonin-like effect on trypanosomes and inhibition of protein kinase C, nucleic acid polymerases and nuclear DNA topoisomerase II. Strong plasmatic protein binding and virtually nonexistent metabolism allows detection of suramin in the body for up to three months. Intravenous administration of 20 mg/kg every 5 or 7 days to a maximum of 5 doses is the usually recommended treatment regimen. Idiosyncratic reactions are seen in about 1 out of 3'000 persons. Collapse, vomiting, shock and eventually death are seen. Administration of a test dose of 4 mg/kg and screening for risk aggravating factors such as onchocercosis, malnourishment and renal insufficiency is strongly advised. Nephrotoxicity with renal function impairment is the main severe adverse effect, but fever, joint pain, pruritus, exfoliative dermatitis, hemolytic anemia, agranulocytosis, jaundice, hepatitis and diarrhea are described. Although efficacious against both subspecies, suramin is nowadays used almost exclusively against *T. b. rhodesiense* infections (reviewed by Nok, 2003) and Fairlamb, 2003).

1.4.2.3 *Diminazene aceturate*

This diamidine, manufactured as a veterinary trypanocidal agent under the name Berenil®, is orally active and has been used in humans on a compassionate basis. The drug is not registered for human use. A major draw-back of Berenil® is the rapid induction of cross-resistance to melarsopro in laboratory infections, making the use of this drug in the clinical setting potentially dangerous (de Koning, 2001).
1.4.3 Chemotherapy of late stage

1.4.3.1 Eflornithine

Eflornithine (DL-alpha-difluoromethylornithin, DFMO, Ornidyl®), originally developed as an anticancer agent, was introduced in the early 1980s and approved by the US Food and Drug Administration for the treatment of late stage *T. b. gambiense* disease in 1990. Eflornithine is an irreversible inhibitor of ornithine decarboxylase that blocks the polyamine synthesis of trypanosomes thus reducing cellular division. The drug penetrates to a certain amount into the CNS, but relapses may be related with insufficient amounts of this trypanostatic drug in the CNS. Large scale use of DFMO is limited by inactivity against at least 50% of *T. b. rhodesiense* isolates, due to the innate lack of susceptibility of this parasite based on a higher ornithine decarboxylase turnover. Adverse events with this drug include convulsions (7%), gastrointestinal symptoms like nausea, vomiting and diarrhea (10%-39%), bone marrow toxicity leading to anemia, leucopenia and thrombocytopenia (25-50%), hearing impairment (5% in cancer patients) and alopecia (5-10%). Currently recommended schedules require 4 daily intravenous infusions for 14 days (400mg/kg/day for adults, 600 mg/kg/day for children). Eflornithine is reasonably active when administered orally and efforts are currently under way to determine the feasibility of using this route of administration in the treatment of HAT. The systematic use of eflornithine is presently very difficult to implement under the severely limited logistic conditions prevailing in the majority of HAT treatment centers. Furthermore, eflornithine is costly and complex to synthesize and produce, meaning that the drug-associated cost of a single patient treatment is estimated in the USD 350 range (reviewed by Burri and Brun, 2003).

1.4.3.2 Melarsoprol

Melarsoprol (Mel B, Arsobal®) is the combination of melarsen oxide with a metal chelator, dimercaprol (British Anti Lewisite, BAL). Trypanocidal action is related to inhibition of trypanosomal glycolytic pathway enzyme phosphofructo-kinase, and interaction with trypanothione, the equivalent of human
glutathione present in trypanosomes. Due to its almost complete insolubility in all commonly used solvents, it is marketed exclusively as a 3.6% solution in propylene glycol in 5-ml ampoules. The characteristics of this solvent make strict intravenous administration mandatory.

Melarsoprol is still the first-line drug for both Rhodesiense and Gambiense late stage HAT. Despite many years of use, data on the pharmacological and pharmacokinetic properties of melarsoprol is limited. The inexistence of specific tests for measuring melarsoprol levels was an important impeditive until recently. Toxicity of the drug also makes studies in healthy volunteers ethically difficult to perform. Melarsoprol (as well as the other presently used drugs) was introduced before the current strict rules for drug approval were implemented: treatment schedules were established on a trial and error basis. Generally treatment is very lengthy, lasting 25-35 days. Treatment regimens for melarsoprol vary considerably depending on the parasite and on the experience with this drug in a given region. Four therapeutic schedules are described by the World Health Organization. They consist of 3 to 4 series of one daily melarsoprol injection for 3 or 4 days, with one-week intervals between the series. Usually melarsoprol dosage is progressively increased to 3.6 mg/kg, and a maximum of 5.0 ml per injection. Although highly efficient in any stage of HAT, the present use of melarsoprol is limited to neurological disease, due to the high toxicity and to the existence of better tolerated drugs for early stage disease.

Adequate pharmacokinetic studies on melarsoprol were performed only recently, thanks to the development of a specific High Performance Liquid Chromatography (HPLC) method (Ericsson, Friden et al., 1994), (Ericsson, Schweda et al., 1997) and a bioactivity assay (Burri and Brun, 1992) for determination of melarsoprol and melarsoprol metabolites in body fluids. These two techniques were combined with the atomic absorption spectrophotometry method for measurement of the arsenic content and pharmacokinetic studies were performed in blood and CSF in humans and in
the monkey model (Burri, Baltz et al., 1993), (Burri, Onyango et al., 1994). A discrepancy between HPLC melarsoprol levels and trypanocidal activity determined by bioassay was observed, indicating that melarsoprol is transformed into metabolites with trypanocidal activity (Bronner, Brun et al., 1998). Melarsoprol biotransformation studies were also performed in rats and in vitro (Gregus and Gyurasics, 2000), (Keiser and Burri, 2000). Melarsoprol has a short half-life of 30 minutes is rapidly converted into melarsen oxide, which is the predominant active metabolite. Melarsen oxide decays with a half-life of 3.9 hours and is covalently bound to plasma proteins. Elimination is via hepatobiliary transport and (in the animal model) appears to be partially glutathione (GHS) dependent. The mean terminal elimination half-life of melarsoprol determined by trypanocidal activity by bioassay is approximately 35 hours. Drug levels in the CSF are generally very low (about 50 times lower than those in serum), never exceeding 10% of the serum levels.

These pharmacokinetic studies led to the introduction of an abridged scheme for melarsoprol application, with a lower total dose, consisting in a 2.2 mg/kg daily dose for 10 consecutive days. A comparative clinical trial in Angola showed that the efficacy and safety of the new scheme were identical to the conventional scheme (Burri, Nkunku et al., 2000). A large multinational clinical trial confirmed these findings and showed that better compliance and cost reduction are the main advantages of this 10 day scheme (Schmid, Richer et al., submitted for publication). The abridged scheme has been recommended during the 27th Meeting of the International Scientific Trypanosomiasis Research Council in late 2003 as the standard treatment for Gambiense late stage disease.

Melarsoprol administration is associated with a number of severe adverse events. Exfoliative and maculopapular skin reactions, polynuropathies, tachycardia, fever, abdominal pain, diarrhea, vomiting, pruritus, chest pain and headache are frequently observed. In addition, propylene glycol produces
painful severe tissue necrosis if injection is not strictly intravenous. Thrombophlebitis and vein fibrosis is common during and after the usual therapeutic schedules (Van Nieuwenhove, 1999). The encephalopathic syndrome is the most important complication of melarsoprol usage and seriously limits this drug overall efficacy.

Interest in arsenical compounds, aside from HAT treatment, lays in their antitumoral activities. Inorganic arsenic trioxide can produce complete remissions and longer survival in patients with acute promyelocytic leukaemia (Kitamura, Yoshida et al., 1997) (Soignet, Maslak et al., 1998) (Niu, Yan et al., 1999). Organic arsenicals are generally considered less toxic than the inorganic compounds. Melarsoprol has been tried in a small number of conventional chemotherapy refractory leukaemia patients with limited success and severe toxicity (Soignet, Tong et al., 1999). Melarsoprol alone or in combination with all-trans-retinoic acid is active against human breast cancer and human prostate cancer cell lines in vitro and in vivo (immunodeficient mice model) and is considered a potentially interesting adjuvant therapy for these conditions (Koshiuka, Elstner et al., 2000).

1.4.4 Patient management

Early stage patients may be managed in the village or as out-patients but late stage patients must be managed in the hospital. Screening tests for anemia, other concomitant blood or intestinal parasitosis and renal impairment are recommended before specific chemotherapy is established (WHO, 1986).

Additional laboratory tests for evaluation of hematologic and biochemical parameters are potentially useful in patient evaluation and management but are seldom available in the setting where HAT is treated. Electrocardiogram, electroencephalogram, polysonnographic recordings, and imaging methods (especially of the CNS) are also useful in patient management but access to these tools is severely limited in most HAT treatment centres.
1.4.4.1 Patient preparation

Before patients receive melarsoprol, the general and nutritional status is improved by the administration of caloric, macro and micronutrients supplementation. Correction of severe anemia is indicated and may require blood transfusion. Fluid and electrolyte disturbances, more frequently observed in severely ill Rhodesiense HAT patients, must be corrected. Concomitant infections should be diagnosed and treated. A variety of interventions mainly aimed at the prevention of melarsoprol-related adverse events is proposed and differently implemented in HAT treatment centres. This patient preparation usually implies postponing melarsoprol therapy, generally for one week (WHO, 1986).

1.4.4.2 Assessment of cure and follow-up

The ideal test allowing precise cure assessment at the end of treatment remains to be developed. Antigen and PCR techniques for trypanosome DNA detection (in CSF) may constitute useful tools for this purpose, but these methods are cumbersome and expensive and no method has yet been validated. Trypanosomal DNA persistence after efficient treatment may also be a problem (Truc, Jamonneau et al., 1999). Differentiation between refractoriness and reinfection in active transmission foci is impossible with the currently available methods.

Ideally patients should be parasitological and clinically checked at discharge, and 1 month, 3 months and 6 months later. Follow-up visits should be made every six months until two years are completed. Identification of trypanosomes in blood or lymph aspirate during follow-up of early stage patients makes lumbar puncture mandatory. For late stage patients lumbar puncture is required for cure assessment (WHO, 1986). However, since normalization of CSF cyto- and biochemical parameters after melarsoprol may be slow, lumbar puncture is not useful before at least six months. Increased CSF cell count or protein content alone are not a valid indication of therapeutic failure as these parameters may actually increase or remain
abnormally high after melarsoprol administration (“l’orage rachidien”) (Janssens, 1977). The existence of worsening neurological signs and symptoms appears to constitute a better indication of drug failure. Trypanosome detection in CSF during follow-up is definitive proof of treatment failure or reinfection and implicates re-treatment.

According to the WHO, a patient is presently considered cured if at the end of a two-year follow-up period no trypanosomes are detected in any body fluid and CSF parameters remain normal or stable. The follow-up coverage is generally below 30% at the end of this two-year period in most treatment centers. Better tools for assessment of cure and follow-up are obviously needed.

1.4.4.3 Treatment failure

The global efficacy of pentamidine and suramin are difficult to evaluate. Despite extensive use of pentamidine (including prophylactic six-month interval administrations), resistance is seldom reported. Patients progressing to late stage disease after pentamidine have been described. Whether this reflects resistance or an initial incorrect staging is difficult to access (WHO, 1989).

Melarsoprol therapeutic failures are being described with increasing frequency. Until recently, the rate of treatment failures with this drug was fairly constant, between 1 to 10 %. In limited regions of Angola, Democratic Republic of Congo, Uganda and Sudan the situation is reaching dramatic levels, with 25%-30% of treated patients being refractory to melarsoprol (Burri, 2001), (Matovu, Enyaru et al., 2001), (Moore, 2001). The exact mechanisms for therapeutic failures and relapses are badly understood (Brun, Schumacher et al., 2001).

Patients treated once with melarsoprol tend to be refractory to further courses of the arsenaclip and should be treated with eflornithine or nifurtimox, alone or in combination (Van Nieuwenhove, 1999). Since nifurtimox is
presently not available, up to more than 25% of patients are left with no option after melarsoprol fails, except that of being enrolled in the growing “waiting lists” for efloremite therapy (personal observation, DRC, Kasai Oriental Province).

1.4.4.4 Alternative (rescue) chemotherapy

1.4.4.4.1 Nifurtimox

Nifurtimox (a 5-nitrofuran, Lampit®) is a cheap oral drug licensed for Chagas disease, which has been used mainly on a compassionate basis to treat melarsoprol or efloremite refractory late stage Gambiense HAT patients. The mode of action is not fully elucidated, probably involving reduction of superoxide, hydrogen peroxide and free radicals production. Anorexia, gastrointestinal and neurological adverse events are very common and dose-related. Reported efficacy and safety data vary greatly in the published small-scale clinical trials (Van Nieuwenhove, 1999). Nifurtimox is to be withdrawn from the American market due to increasing rates of resistance observed in Chagas disease (Buguet, Bourdon et al., 2001). Efforts are under way to expand the product license for use in HAT, so that correctly powered safety and efficacy clinical trials can be performed (Jannin, personal communication).

1.4.4.4.2 Combination therapy

Despite several anecdotic reports and small scale clinical trials showing efficacy for almost any combination of the available and alternative drugs, combination therapy awaits for confirmation of in vitro and in vivo synergisms so that correctly powered clinical trials for scheme optimization may be performed (Keiser, Stich et al., 2001). Combination therapy theoretical advantages include: delay in resistance emergence, increased treatment efficacy and cure rates as well as reduced single-drug dosage and shorter therapeutic schemes, possibly resulting in reduced toxicity.
1.4.4.5 Treatment complications

The care for a final stage patient is complicated by the frequently existing difficulties encountered in convulsion control, bacterial infection treatment and in the implementation of measures to support a comatose patient. The human resources and the medical equipment and consumables, including additional drugs necessary for management of critically-ill patient are frequently lacking in many HAT treatment centres (personal observations, Angola and DRC).

The encephalopathic syndrome is well known and feared by both medical personnel and patients as the most severe and important meilarsoprol-associated complication of HAT treatment.

1.5 The encephalopathic syndrome

The encephalopathic syndrome (ES) is a life-threatening event taking place during or immediately after treatment of a patient with meilarsoprol. It results in a dramatic deterioration of the patient’s neurological status. Our ignorance on the nature of ES is demonstrated by the synonyms applied to the syndrome, which include arsenical encephalopathy, reactive encephalopathy, hemorrhagic encephalopathy, post-treatment reactive encephalopathy and toxic encephalopathy. Some authors have distinguished three forms or types of ES. Distinction between the forms is based on clinical parameters and on a few anatomopathological studies performed in ES patients (Manuelidis E.E, Robertson D.H et al., 1965), (Haller, Adams et al., 1986), (Adams, Haller et al., 1986) (Table 3).

The first form consists in a progressive state of severe psychic and verbal agitation, mental confusion and disorientation. The prognosis is usually good and the psychotic changes resolve after a few days. The second type is marked by the sudden onset of epileptiform convulsions that may progress to status epilepticus. The response to supportive therapy is poor. The patient may slip into a coma and die. The third type consists in the development of a deep
coma, within hours, very often followed by death (Haller, Adams et al., 1986), (Blum, Nkunku et al., 2001). Overlapping between these forms apparently occurs. It is not clear whether they represent different manifestations of the same phenomenon or have different etiologies.

<table>
<thead>
<tr>
<th>Hypothetical forms of the encephalopathic syndrome</th>
<th>Clinical and anathomopathological features</th>
<th>Prognosis</th>
</tr>
</thead>
</table>
| **Convulsive**                                    | Isolated or repeated epileptiform convulsions  
Acute cerebral oedema, CSF pressure elevated  
Hypoxic brain damage                                    | Bad, at least half of the patients die          |
| **Comatose**                                     | Progressive coma  
No signs of cerebral oedema, CSF pressure normal  
Acute hemorrhagic encephalopathy                | Very bad, death in most cases                 |
| **Psychotic reactions**                          | Aggressive behaviour, confusion, disorientation  
No anatomopathological data available            | Good, survival in most cases                  |

Table 3: Characteristics of the hypothetical forms of the encephalopathic syndrome

ES is said to occur in 2 to 10% of all melarsoprol treatments and the overall mortality of the second and third types of ES is said to be in the 50-100% range (Sina, Triolo et al., 1977). Therefore, treating a patient with this drug means to accept an unusually high treatment-related fatality rate for an infectious disease (Coulaud, Vachon et al., 1975).

The presently accepted hypotheses for the etiology of ES have an immunological background. The main working hypotheses are indicated in table 4.
<table>
<thead>
<tr>
<th>Proposed etiologies for the encephalopathic syndrome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune complex deposition</td>
<td>Lambert, 1981</td>
</tr>
<tr>
<td>Parasite antigen in the CNS (glial cells) became targets of antibodies and/or T cell destruction</td>
<td>Pepin, 1991</td>
</tr>
<tr>
<td>Subcurative treatment followed by immune reaction in the CNS</td>
<td>Hunter, 1992</td>
</tr>
<tr>
<td>Direct arsenical toxicity</td>
<td>Soignet, 1999</td>
</tr>
<tr>
<td>Misdirected immune reaction initiated by haptens formed by MelB metabolites + plasma proteins</td>
<td>Keiser, 2000</td>
</tr>
</tbody>
</table>

Table 4: Working hypothesis for the etiology of the encephalopathic syndrome

The published literature on ES usually describes only small series of patients. Differences in methodologies and the significant geographic and temporal scattering of clinical studies are prone to introduce several analytical biases. The homogeneity of diagnostic criteria for ES is not warranted. The differential diagnosis of ES is not always distinctive, as the clinical manifestations of the syndrome may be similar to those of HAT itself and/or to those of concomitant diseases, especially those affecting the CNS.

ES appears to be an unpredictable phenomenon. Evidence for risk factors for the development of ES comes from few correctly powered clinical studies. Results are not consistently reproduced and cannot be considered solid or robust.

In the absence of a known etiology, a variety of empirically designed interventions have been used in the prevention and management of ES. The precise value of these interventions also remains to be correctly defined in correctly powered clinical studies.
2 JUSTIFICATION, GOAL AND OBJECTIVES OF THIS PROJECT

2.1 Justification
HAT has re-emerged in sub-Saharan Africa. At least 60 million individuals are at risk of acquiring the infection and 66,000 are dying every year. If not efficiently treated sleeping sickness virtually kills every one infected. The main burden of disease is associated with the Gambiense form of trypanosomiasis.

Control of HAT transmission is possible and has been obtained in the past. Diagnosis and determination of the stage of disease followed by adequate drug treatment is crucial for individual treatment and for disease control by means of human reservoir sterilisation in Gambiense trypanosomiasis. Optimized old methods and strategies and newly developed ones can bring control of Gambiense disease and are considered cost-effective.

HAT is a neglected disease. The limited available funds are badly needed for surveillance and control activities and for human and logistic resource capacity building. Available drugs for efficient treatment of HAT are presently severely limited. Very little drug development is currently taking place. Optimization of the chemotherapy of sleeping sickness with the presently available drugs is thus a priority.

Melarsoprol is and will remain for many years the first choice for treatment of both forms of late stage HAT. Rhodesiense late stage patients can presently be efficiently treated only with this drug. Melarsoprol administration is associated with the encephalopathic syndrome, a badly defined life-threatening and unpredictable neurological complication. With a 2-10 % incidence in all melarsoprol treatments, ES jeopardizes the global efficacy of this drug, killing at least 50% of those affected.

Existing literature data on the definition, etiology, risk factors and value of preventive or therapeutic interventions on ES is limited in quantity and quality. Studies often show conflicting results. In order to reduce the
incidence and mortality of the encephalopathic syndrome valid evidence-based concepts are needed, to be able to correctly define, diagnose, predict, avoid, and efficiently treat the phenomenon.

The systematic review methodology, developed by the Cochrane Group, has been shown to be useful in clarifying complex medical issues. This method can help identify biases and methodological flaws in the available literature data on a given subject. Clinicians may obtain a clearer picture on the validity of evidence available. This methodology may assist in the establishment of scientifically correct definitions and on the validity of preventive and therapeutic interventions in ES and may indicate directions for future studies.

The cause of ES is generally assumed to be an immune phenomenon occurring mainly in the CNS and involving a complex and peculiar host-drug-parasite interaction. The Human Leukocyte Antigen (HLA) complex is presently accepted as a major determinant of the immune response in humans. HLA determines the differentiation between self and non-self and is involved in auto-immune disease as well as in determining susceptibility and resistance to several infectious diseases. New molecular methods based in DNA amplification by the polymerase chain reaction and allele detection by specific probes are presently available that allow a more precise determination of Class I and Class II haplotypes. The HLA type may be a determinant of ES susceptibility.

2.2 **Overall Goals**

To contribute to the acquisition of knowledge on the definition, etiology, and risk factors of the melarsoprol-related encephalopathic syndrome in HAT.

To determine the validity of the preventive and therapeutic interventions used in the melarsoprol-related encephalopathic syndrome in HAT.
2.3 **Objectives**

- To perform a systematic review of the published and unpublished literature data on the melarsoprol-related encephalopathic syndrome in HAT, aimed at:
  
  - Improving the definition and consequently the diagnosis of ES
  
  - Evaluating the effectiveness of the presently proposed preventive and therapeutic interventions in ES
  
  - Identifying potential risk factors for ES and assessing their role
  
  - Establishing the basis for the discussion on the etiology of ES

- To design and perform a prospective case-control clinical study on the encephalopathic syndrome in melarsoprol-treated late stage Gambiense HAT patients aimed at:
  
  - Determining the clinical characteristics of ES
  
  - Establishing the existence of clinical risk factors for ES
  
  - Evaluating the existence of an association between ES and the HLA type
PART II:
SYSTEMATIC REVIEW ON THE ENCEPHALOPATHIC SYNDROME

3 INTRODUCTION

3.1 RATIONALE

Solid evidence is missing on many aspects of the encephalopathic syndrome in melarsoprol treated HAT patients. Critical concepts like the definition and etiology of ES are deficiently established. The search for risk factors for ES has so far yielded conflicting data and has not been conclusive. However, clinicians still need valid evidence on the role of the multiple preventive and therapeutic interventions presently in use. Presently and probably in coming years no drug alternatives for second stage HAT are or will be easily available. Additionally, if melarsoprol is ever to find a place in treatment of oncological diseases, knowledge on the etiology, risk factors, prevention and management of this life-threatening melarsoprol-associated event has to be improved.

3.1.1 The Cochrane collaboration Group Methodology for systematic reviews

The methodology for systematic reviews was originally developed by members of the Cochrane Collaboration Group, as a tool to provide scientific evidence for the medical decision process on complex and critical therapeutic issues. Systematic reviews are scientific investigations that use planned methods and follow precise methodological rules to extract the results of multiple individual original studies and to synthesize them in order to limit bias and error. When a systematic review is applied to controlled randomized trials, the results can be quantified and statistic tools can be used for analysis: this is known as a meta-analysis A successful meta-analysis should increase power and precision, provide an overall estimate of range of effect and identify greater-than-expected variability among study results (heterogeneity) (Imperiale, 1999).
The use of this methodology for studies that are not controlled randomized clinical trials is hampered by the heterogeneity of the data in such studies and subsequent difficulties in developing adequate statistical methods to deal with this. Nevertheless, the use of a structured approach in relationship to biomedical issues may help overcome the common problems often observed in conventional reviews (Clarke and Oxman).

Several important problems have been identified that interfere with the quality of a systematic review. A central issue relates to which studies are included or excluded. Problems can be avoided if data extraction avoids some well-known and defined biases.

The term publication bias relates to the fact that many studies that suggest a negative or indifferent outcome of a given treatment never get published. In this situation, a review that includes only published data would identify a spurious beneficial treatment (Easterbrook, Berlin et al., 1991).

English language bias (or Tower of Babel bias) refers to the fact that many reviews are based only on trials published in English. Many methodologically correct trials are indeed published in other languages, and their exclusion introduces a bias (Grégoire, Derderian et al., 1995).

Incomplete search bias applies to the inadequacy of the efforts that have been made to locate reports of studies. The efficacy of reviews performed on electronic literature databases alone (such as Medline) depends on the adequacy of indexing and search strategies of the databases. Alternative methods such as hand search are time consuming but can retrieve all available evidence on a given theme (Dickersin, Scherer et al., 1994).

The bias derived from checking the opinion of experts on a given field and checking the reference lists of other studies is called the citation bias. A tendency to locate supportive studies rather than those with a negative
outcome (regardless of the quality of the study) is observed if reference lists are used. This can bias the findings of a systematic review (Ravnkov, 1992).

A multicenter study with significant results is more likely to be give rise to multiple publications, which makes more likely that they are located and included in the systematic review. It may be very difficult for reviewers to determine if two papers represent duplicate publications of a trial or not. This is called the multiple publication bias (Huston and Moher, 1996).

Provision of data bias relates to the impossibility to retrieve additional data from the investigators. This tends to occur if the results of a study indicate an unfavorable direction.

Clinical data about HAT and ES has been obtained over a long period of time and from very different settings, often under difficult circumstances due to the rural nature of the disease. In fact, many clinical studies include a very limited number of patients and are often compassionate studies. In HAT and in particular in ES, carefully planned randomized controlled trials are not the rule but the exception.

Important clinical or experimental data is often presented only during scientific meetings, and is cited as “personal communication” or published in abstract books. Furthermore, many physical difficulties exist in communications which impair data sharing between health professionals in the field, the national trypanosomiasis control programs and central agencies.
3.2 Objectives

The purpose of the present study was to perform a systematic review on published and unpublished literature on the encephalopathic syndrome during treatment of HAT with melarsoprol.

The main goals were to provide clinicians with a more precise and consistent definition of the syndrome and to assess the effectiveness of interventions for the prevention and management of ES. Additional aims were to identify potential risk factors for ES and to establish the basis for the discussion on the etiology and pathogenesis of the phenomenon.
4 MATERIALS AND METHODS

4.1 STUDY PROTOCOL AND MEASURED OUTCOMES

A systematic review (SR) protocol based on the methodology developed by the Cochrane Collaboration Group was elaborated. The protocol used included the rationale of the SR, the objectives, and the methods to locate, select and critically evaluate datasets. The primary outcomes measured were the occurrence of ES and death associated with an ES episode (case fatality rate, CFR). Secondary outcomes included the duration of the ES episode, and the result of melarsoprol resumption following an ES.

4.2 DEFINITION OF THE ENCEPHALOPATHIC SYNDROME

For the purpose of our study, ES was defined as the sudden deterioration of the patient’s neurological status, characterized by the development of convulsions and/or coma and/or an acute and persistent psychotic alteration in mental status, during or immediately following melarsoprol treatment. Events reported by the authors of each dataset as an ES episode were compared to this definition. To increase the precision of the definition of ES, the existence of convulsions, changes in the patient’s level of consciousness, or mental changes of the psychotic type previous to melarsoprol administration was checked for each dataset.

4.3 SEARCH STRATEGY

The search included electronic and printed bibliographic databases, “grey” literature (technical reports, conference proceedings, and dissertations), unpublished or ongoing clinical trials and standard textbooks of tropical medicine. There were no language limitations. The lower temporal limit for the search was 1949, the year of introduction of melarsoprol to clinical practice. Accessed electronic databases included Medline, Oldmedline, Embase, Biosis, and Cochrane Central. A manual search was performed on two experts’ databases on HAT, plus the WHO database on sleeping sickness. Search terms included combinations of keywords that were pre-tested in order to produce the maximum number of relevant hits. The
searches in Embase and Biosis were performed with the assistance of an expert librarian.

Around 2500 documents were primarily scanned for relevance. This was accomplished by considering the title and/or abstract of electronically retrieved documents and the full text for those manually examined. The citation lists of the relevant documents were searched for additional references. A preliminary database in EndNote® (Thomson ISI ResearchSoft, USA) consisting of 298 records was obtained.

4.4 INCLUSION AND EXCLUSION CRITERIA

Original studies reporting the human therapeutic use of melarsoprol for treatment of HAT and the subsequent development of an encephalopathic syndrome were included. Included studies could be of any type of investigational methodology and include patients of any gender, age, ethnicity, setting, country or region. Records in the preliminary database were screened for predefined inclusion criteria by one of the reviewers, using a “Record Sheet for Eligibility Criteria”. A second reviewer crosschecked the eligibility of studies. Excluded studies were listed and the reason for exclusion stated.

Textbook chapters and review papers, reports on the development of an encephalopathic syndrome (or similar phenomena) associated with drugs other than melarsoprol and studies reporting the use of melarsoprol for diseases other than HAT were excluded from the analysis but were retained for the discussion and used for citation chasing.

4.5 DATA EXTRACTION AND DATASET CLASSIFICATION

A Microsoft Access® database was built for insertion of all the parameters extracted from selected studies. Extracted parameters were as follows: characteristics of datasets and of the participants, form of HAT being treated, treatment schedule for melarsoprol, clinical features of the encephalopathic syndrome, interventions for the prevention and management of the syndrome and final outcome. Severe adverse effects and events other than ES were also
recorded. Abstracts were created if not available and inserted into the Microsoft Access® database.

Datasets were classified according to their contents. Categories for datasets are described in Table 5. Datasets were considered duplicates when the same group of patients was described in two or more documents. In this case, the dataset giving the highest level of clinical details was retained. If the same set of patients was described in one dataset but subsequently complemented with additional cases in another publication, the latter was selected, but valuable clinical data from both was preserved.

4.6 DATA ANALYSIS AND SYNTHESIS

Due to the considerable heterogeneity of the studies, the possible approach to data synthesis was descriptive and qualitative rather than quantitative. Case reports and particular issues were considered on a case-to-case basis. The “best and worst case scenario” analysis approach was used when appropriated.

Clinical trials, case reports and case series were separated according to the form of sleeping sickness described in the study (Gambiense or Rhodesiense). To avoid major bias, case reports and case series describing less than 4 ES patients were excluded from the calculations of the incidence risk (i.e. the probability that a person initially free from the disease develops it at some time during the period of observation) and of the CFR for ES. Studies exclusively describing a selected population of ES patients were also excluded from the determination of the incidence risk but were included for calculation of the case fatality rate.

Specific issues, such as the clinical characterization of ES or the role of interventions for prevention and management of ES were addressed by selecting datasets where the desired parameters and the primary outcomes (occurrence of ES or death from ES) were simultaneously available.
To study the role of preventive and therapeutic interventions in relation to the incidence risk of ES and to ES case fatality rate, a logistic regression was performed using Intercooled Stata 7.0 (Stata Corporation, USA).
5 Results

5.1 Dataset characterisation and classification

The search yielded a total of 157 references considered potentially relevant, i.e. referring to an encephalopathic syndrome or a similar phenomenon independently of the drug used, of the disease being treated and of the type of investigational methodology of the reference. Thereof a hundred and thirty (85.5%) datasets were published. After screening for inclusion criteria, 111 references were excluded (Table 5).

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of datasets</th>
<th>Status for analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical trials and case controlled studies</td>
<td>10</td>
<td>Accepted</td>
</tr>
<tr>
<td>Case series</td>
<td>33</td>
<td>Accepted</td>
</tr>
<tr>
<td>Case report</td>
<td>03</td>
<td>Accepted</td>
</tr>
</tbody>
</table>

**Published vs. unpublished: 35/11 (76%)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of datasets</th>
<th>Status for analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reviews, book chapters, thesis, or ES cited</td>
<td>31</td>
<td>Refused</td>
</tr>
<tr>
<td>Physiopathology studies</td>
<td>25</td>
<td>Refused</td>
</tr>
<tr>
<td>Animal model</td>
<td>13</td>
<td>Refused</td>
</tr>
<tr>
<td>Duplicate set of patients</td>
<td>11</td>
<td>Refused</td>
</tr>
<tr>
<td>Not Melarsoprol not HAT</td>
<td>08</td>
<td>Refused</td>
</tr>
<tr>
<td>HAT but not Melarsoprol</td>
<td>06</td>
<td>Refused</td>
</tr>
<tr>
<td>Atypical cases</td>
<td>05</td>
<td>Refused</td>
</tr>
<tr>
<td>Anatomopathological studies</td>
<td>05</td>
<td>Refused</td>
</tr>
<tr>
<td>Clinical research</td>
<td>04</td>
<td>Refused</td>
</tr>
<tr>
<td>Melarsoprol but not HAT</td>
<td>01</td>
<td>Refused</td>
</tr>
<tr>
<td>Letter</td>
<td>01</td>
<td>Refused</td>
</tr>
<tr>
<td>Data extraction impossible</td>
<td>01</td>
<td>Refused</td>
</tr>
</tbody>
</table>

**Published vs. unpublished: 95/16 (85.5%)**

**Sub-total: 111**

**Total: 157**

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Table 5: Classification of datasets according to status in systematic review
Under the “atypical cases” category we classified case reports describing difficulties in the management of HAT associated with melarsoprol toxicity or concurrent diseases. “Clinical research” references include the use of uncommon and potentially valuable diagnostic methods (Electroencephalography, Magnetic Resonance Imaging) in HAT encephalitis and a study on the febrile reactions to melarsoprol (with and without concomitant steroid) administration.

Among the relevant datasets, the number of clinical studies referring to the use of melarsoprol in HAT and subsequent development of an ES episode and thus accepted for analysis was 46. Case series and case reports (n = 36) accounted for 76 % of the accepted references. Only 10 datasets were clinical trials or case controlled studies. The distribution of the accepted datasets according to the form of HAT is shown in Table 6.

<table>
<thead>
<tr>
<th></th>
<th>Gambiense</th>
<th>Rhodesiense</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case report</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Case Series</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>Clinical Trial</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 6: Classification of accepted datasets according to the form of HAT

The total number of patients with HAT, the total number of patients treated with melarsoprol, and the total number of ES episodes (for each form of sleeping sickness) identified in the accepted studies are shown in Table 7. One unpublished dataset describing 3165 HAT patients was discarded, because the number of patients receiving melarsoprol is not given (Mbulamberi, 1987). An additional publication where the described cases were “mainly due to Trypanosoma rhodesiense” was also not used for the calculation (Robertson, 1963).
<table>
<thead>
<tr>
<th></th>
<th>Total Number of Patients with HAT</th>
<th>Total Number of Patients treated with Melarsoprol</th>
<th>Total Number of Patients with ES</th>
<th>Incidence of ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gambiense</td>
<td>12478</td>
<td>8772</td>
<td>408</td>
<td>4.65</td>
</tr>
<tr>
<td>Rhodesiense</td>
<td>3165</td>
<td>1934</td>
<td>154</td>
<td>7.96</td>
</tr>
<tr>
<td>Total</td>
<td>15643</td>
<td>10706</td>
<td>562</td>
<td>5.25</td>
</tr>
</tbody>
</table>

Table 7: Total number of patients in accepted datasets

5.2 Epidemiology of the Encephalopathic Syndrome

The number of datasets where the overall HAT mortality during treatment and the case fatality rate for the ES episode is available, thus allowing calculation of the percentage of deaths due to ES is limited. These indicators are shown in Table 8 and 9 for the Gambiense and the Rhodesiense forms respectively. For Gambiense the table includes 13 datasets where overall mortality and case fatality rate for ES are available and 8 additional datasets where only case fatality rate for ES episode is available. For Rhodesiense the corresponding numbers of datasets are 7 and 3. Case Reports and case series with less than 4 ES patients described were excluded. The incidence risk for ES was recalculated for the datasets included, and maximum, minimum, average and median values are shown.
Table 8: Gambiense disease: number of patients in selected datasets

<table>
<thead>
<tr>
<th>Incidence risk of ES</th>
<th>HAT mortality</th>
<th>Deaths due to ES</th>
<th>CFR of ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Maximum</td>
<td>23.5</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.5</td>
<td>2.7</td>
<td>13.7</td>
</tr>
<tr>
<td>Average</td>
<td>4.3</td>
<td>9.4</td>
<td>46.4</td>
</tr>
<tr>
<td>Median</td>
<td>5.6</td>
<td>6.3</td>
<td>44.4</td>
</tr>
</tbody>
</table>

Table 9: Rhodesiense disease: number of patients in selected datasets

<table>
<thead>
<tr>
<th>Incidence risk of ES</th>
<th>HAT mortality</th>
<th>Deaths due to ES</th>
<th>CFR of ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Maximum</td>
<td>28.0</td>
<td>19.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.5</td>
<td>5.2</td>
<td>6.6</td>
</tr>
<tr>
<td>Average</td>
<td>10.6</td>
<td>11.6</td>
<td>42.2</td>
</tr>
<tr>
<td>Median</td>
<td>10.6</td>
<td>11.5</td>
<td>46.0</td>
</tr>
</tbody>
</table>

5.2.1 Incidence Risk

For both forms of sleeping sickness, the incidence risk for ES and the number of patients treated with melarsoprol in each of the 27 studies (19 for *T. b. gambiense*; 8 for *T. b. rhodesiense*) that permitted to calculate this proportion are shown in Figure 7. The lowest incidence risk for ES is 1.5% for both forms of the disease whereas the highest risk is 23.5% for Gambiense and 28% for Rhodesiense trypanosomiasis. The calculated correlation coefficient is shown ($R^2$).
Figure 7: Geographical distribution of the Incidence risk for ES (bars), according to the country of origin of patients and the number of patients treated with melarsoprol (○). Countries with the Rhodesiense form of HAT are marked with (**)..

5.2.2 Case fatality rate
The case fatality rate for ES and the number of patients affected by ES in each study are shown for the Gambiense (18 studies) and the Rhodesiense (10 studies) forms in Graph 2. The lowest CFR is 15.3% for Gambiense and 21.4% for Rhodesiense. CFR of ES reaches 100% in one publication on Gambiense and in two publications on Rhodesiense. Figure 8 shows the distribution of incidence according to the case fatality rate for ES. The calculated correlation coefficient is shown (R²).
5.2.3 Seasonal fluctuation

A seasonal variation on the incidence and mortality of ES was described by the MSF HAT control program in North Western Uganda, Moyo district, in patients with the Gambiense form of HAT. During the observation period of 1987 to 1999 a consistent increase in the incidence of ES from 3% in the first semester to 10% in the second semester, especially between June and October of each year, was detected. This higher seasonal incidence was also associated with a higher ES case fatality rate. Furthermore, in 1992 and 1993 a significant increase in the number of ES cases in patients admitted between August and September is observed. Following extensive epidemiological investigations, no single factor could be associated with the epidemic outbreak of ES during those periods. The existence of exogenous co-factors such as infectious or nutritional factors was proposed (Barret, 1993), (Ancelle,
Barret et al., 1994). However, this type of phenomenon is not described elsewhere.

5.3 Definition of the Encephalopathic Syndrome

Datasets were classified according to the presence or absence of our pre-established definition criteria for ES. In 30 out the 46 accepted datasets we could find a definition of ES, whether established by the authors or as part of the description of the cases. In 14 datasets no definition whatsoever of ES was given or obtainable.

A definition of ES that included a normal level of consciousness on patient’s admission was given in 18 studies. Among these, we identified 12 studies (5 clinical trials) that distinguished the 3 types of manifestations of ES (coma, convulsions, mental disturbance). Psychotic mental disturbances are not considered as a manifestation of ES in 5 case series. Tremors and “lack of evidence for other causes for meningoencephalitis” complemented the definition criteria of ES in one study. In one dataset the existence of fever, headache, conjunctivitis and confusion was considered indicative of ES and the attending physician could launch the therapy of ES. Among the remaining 12 datasets where the patient’s level of consciousness on admission is not specified, 6 datasets describe the 3 types of manifestations of ES, whereas 6 datasets describe the coma or convulsive types isolated.

After excluding case reports and case series with less than 4 patients, 15 datasets (out of 46) with a pre-established definition for ES remain, describing a total number of 221 ES cases. In six (40%) of these datasets we found a mixed convulsion and coma type of ES. The sequence of events is usually convulsions followed by coma. In 3 additional datasets mental changes like confusion, agitation, or apathy prompt for the therapeutic protocol for ES or precede convulsions or coma. A description of isolated acute psychotic changes consistent with an exclusively mental type of ES is found in 4 datasets. Two authors clearly state that the 3 types of ES do not overlap
(Haller, Adams et al., 1986), (Blum, Nkunku et al., 2001). In case reports, ES is characterized as only convulsions (2 datasets) only coma (2 datasets), coma and convulsions (1 dataset) and coma, convulsions and mental changes (1 dataset).

Fever is the most frequently cited sign associated with ES (12 datasets). In some series fever is almost always present preceding or during ES. The existence of fever is frequently associated with a higher case fatality rate. The most frequently found symptom preceding full-blown ES is headache (13 datasets). Nausea, vomiting, dizziness, and tremors are also consistently cited as important initial symptoms and signs, diarrhea being less frequent. In the reviewed literature, conjunctival hyperemia is cited 4 times and was considered an important sign during the IMPAMEL II study in Sudan (Burri, unpublished).

A distinction between the so-called “reactive” and the “hemorrhagic” type of ES was found in 4 original studies. The reactive type is characterized by heralding constitutional signs and symptoms such as fever and tremors, followed by convulsions. The prognosis of this type of ES considered better than the one associated with the hemorrhagic type, which is characterized by the unannounced and sudden onset of progressive coma and death.

5.4 RISK FACTORS FOR THE ENCEPHALOPATHIC SYNDROME

5.4.1 Gender distribution

Gender distribution, incidence risk and case fatality rate for ES are simultaneously available in 10 publications for Gambiense and in 2 publications for Rhodesiense. In Gambiense, the highest incidence risk (14.7%) corresponds to a 44/56 M/F ratio (0.78) and the lowest incidence risk (1.7%) to a 47/53 M/F ratio (0.88). The highest case fatality rate (75%) corresponds to a 56/44 M/F ratio (1.27) and the lowest (15.3%) to a 47/53 M/F ratio (0.88). In Rhodesiense, an incidence of 5.2% with a mortality of 50% corresponds to an M/F ratio of 51/49 (1.04) and an incidence of 18%
with a mortality of 52.6% relates to an M/F ratio of 75/25 (3). In the reviewed literature gender is not considered to play a significant role in the incidence or mortality of ES.

A higher risk of ES in males is found by Pepin et al. in 1989 in the DRC (Pepin, Milord et al., 1989), but this tendency is not confirmed (p value: 0.22) in a subsequent publication with an extended number of patients (n: 1083) by the same author in the same setting (Pepin, Milord et al., 1995).

In the analysis of 3817 HAT cases treated with melarsoprol between 1987 and 1999, summarizing the experience of “Médecins Sans Frontières” (MSF, French Section) in sleeping sickness control activities in North Western Uganda, no particular trend concerning gender and susceptibility towards ES was found (Barboza, 2000). However, when the analysis is restricted to the subset of patients observed during what is considered an epidemic of ES that occurred during 1992 and 1993 in the same setting, male gender is considered as a risk factor for ES (attack rate of ES: 18.7% for males and 4% for females, risk for males 2.27 times higher then for females) (Barret, 1993).

5.4.2 Age distribution
Age distribution, incidence risk and case fatality rate for ES are simultaneously available in 16 studies for Gambiense and in 8 for Rhodesiense. The majority of studies, i.e. 17 out of 24 (70%), describe a mixed population of adults and children. Age is generally considered neither as a risk factor for ES nor associated with a higher ES case fatality rate, but studies that specifically refer to this parameter are few.

Only one published study (a series of 227 melarsoprol treated patients over a 17 years observation period) exclusively describes patients in the pediatric (0-6 year age) range (Triolo, Trova et al., 1985). Incidence risk for ES was 1.7%, with a case fatality rate of 33% (2 deaths). The incidence risk is similar to the one observed in the 7-14 year age range (where the CFR is 16.6%) and approximately half the one observed in the adult population (where the CFR
is 41.6%). The difference between CRFs is not statistically significant (p: 1.0; RR: 0.85, C.I. 95%: 0.24 to 2.98). Interestingly, ES was not observed in children under 3 (the number of patients under age 3 is not available). The authors conclude that, although complicated by difficult technical problems related to the mandatory intravenous administration of the drug, melarsoprol toxicity in children is not more severe than in adults.

In the above mentioned experience of MSF in North Western Uganda, the “Arsobal related Case Fatality Rate” due to “Arsobal Related Encephalopathy” was 4 times lower in children under 10 than in the rest of the population (p<0.001) (Barboza, 2000). Haller, in Ivory Coast, indicates that young children and elderly people are less prone to ES, but no numbers are presented (Haller, Adams et al., 1986).

In contradiction, in a series of 64 ES cases, a non-significant trend towards more ES in patients over 65 with a higher case fatality rate was found (Pepin, Milord et al., 1995).

In the preliminary analysis of 2020 patients treated with the new concise schedule for melarsoprol (IMPAMEL II) and developing ES (152 patients), age is neither significantly associated with the risk for ES nor with a higher CFR (Schmid, Chappuis et al., submitted for publication). This analysis confirms the previously published results of a series of 38 ES patients observed during the preparatory phase of this study (IMPAMEL I), in Angola (Blum, Nkunku et al., 2001).

For the Rhodesiense form of sleeping sickness, Veeken et al. described 19 ES cases in Tanzania. Patients below age 18 did not develop ES. Four out of the 5 patients over age 40 developing ES died (Veeken, Ebeling et al., 1989).

Triolo describes what is “possibly the first and only case of congenital HAT and ES”. Both mother and child were diagnosed in first stage HAT, without changes in cerebrospinal fluid (CSF). The mother tolerated 3 injections of
Melarsoprol, but the apparently normal newborn developed convulsions and coma after the second Arsobal® injection and died in 24 hours (Triolo, 1990).

5.4.3 Ethnic group
The nationality or the ethnic group of the participants is specified in 11 studies. In most of them patients belong to several ethnic groups. In the reviewed literature we found no indication for a different incidence or ES case fatality rate in specific ethnic groups.

5.4.4 Clinical status on admission
HAT stage determination is available in 20 studies but the criteria for stage determination are not uniform. Several different classifications for staging are used, according to the presence or absence of trypanosomes, the number of white blood cell (WBC) or protein content in CSF, or the presence or absence of late stage “pathognomonic” neurological signs or symptoms. The threshold for WBC count in CSF for definition of second stage HAT (CNS disease) ranges from 3 to 20 cells/mm³.

Patients may also be classified to be in an advanced or moribund stage, in a good condition or relapsing after initial treatments (“old cases”). This diversity of classifications makes comparisons difficult.

The reviewed literature contains reference of the use of melarsoprol to treat first stage HAT patients, especially during the first 5 years of clinical experience with this drug. ES is also described in first stage patients treated with melarsoprol, but the exact number of patients in this situation is impossible to retrieve from the datasets. Since less toxic alternative drugs exist for first stage disease, the use of melarsoprol in first stage HAT was abandoned, and in the majority of studies (16 out of 20) where a stage definition is given, melarsoprol is used to treat only late stage patients.

In this context, 2 studies show an association between ES and a high cell count in CSF (> 100 cells/mm³), one in Gambiense and two in Rhodesiense
(the study in Gambiense is a compilation of two previous studies by the same author). French authors also describe the dissociation between clinical symptoms and a significantly modified CSF (i.e. few or no symptoms and a good general status and increased cell count or protein content or trypanosomes detected in CSF) as being associated with ES (Antoine, 1977), (Bertrand, Serie et al., 1973). However, these associations are not consistently found in the reviewed literature: 2 (one in Gambiense and one in Rhodesiense) studies do not find a relation between CSF changes and the development of ES. In Gambiense HAT, the presence of trypanosomes in CSF but not in blood or lymph is correlated with a higher risk of ES in one study, while a tendency towards a higher risk of ES is found in patients with trypanosomes in blood or lymph but not in CSF in another study.

The same sort of conflicting observations applies to patients clinically classified with as in “advanced disease” or “moribund” versus patients with few symptoms or patients in a “good condition”. An association between a poor patient condition on admission and ES is described in 4 studies (2 for each form of HAT) but does not exist in 3 other (2 Gambiense and one Rhodesiense).

During the IMPAMEL II trial the Body Mass Index (BMI) was obtained for 2020 patients. No correlation between a low BMI (<16.5, indicating severe malnutrition) and ES was found (p: 0.4; RR: 0.8; CI: 0.6-1.2) in the general study population (Schmid, unpublished data). Malnutrition on admission (defined by the BMI) protects against concomitant infection, in particular malaria (HR: 0.8, p: 0.003 and HR: 0.8, p: 0.0006 respectively) in children (Schmid, Chappuis et al., submitted for publication).

### 5.4.5 Disease form

The incidence risk for ES (average 10.6 vs. 4.3, median 10.6 vs. 5.6) and the ES case fatality rate (average 57.3 vs. 43.8, median 52.6 vs. 50) are higher in Rhodesiense than in Gambiense (see tables 8 and 9). Overall HAT mortality
is also higher for Rhodesiense than for Gambiense (average 11.6 vs. 9.4, median 11.5 vs. 6.3 respectively) but the percentage of deaths due to ES do not significantly differ.

5.4.6 Melarsoprol treatment schedule

The analyzed studies describe a great variety of treatment schedules or protocols using melarsoprol. More than 20 different protocols are used. Differences between schedules include the dose per administration (range: 1.5 to 4 mg/kg/body weight), the number of injections per series (range: 3 to 10 daily injections), the number of series (range: 1 to 4 series), the interval between series (range: 7 to 15 days), and the characteristics of the dosage in the series (increasing or constant dose). Different schedules may be used in the same dataset according to the clinical condition of the individual patient. The number of series is frequently based on the Neujean protocol, which uses the number of cells in the CSF as an indication for the type melarsoprol schedule. Patients with evidence of a more advanced CNS disease are given a larger total dose of melarsoprol.

Schedules using a slowly increasing dosage of melarsoprol are more frequently used in the Rhodesiense form. In this form of HAT, authors tend to be more careful with the initial Arsobal® dose, due to the very frequently observed initial febrile reactions to melarsoprol. The same observation is described in patients with the Gambiense form of HAT admitted in a poor condition.

The enormous between-studies variability in melarsoprol schedule and the pooling together of patients receiving different dosages of melarsoprol in one given study makes determination of the total dose of melarsoprol administered before the appearance of ES impossible.

ES is described at any time point during melarsoprol administration (from the first to the last melarsoprol dose) and up to 30 days after the last administration. Reports describing the occurrence of ES following the first
application of melarsoprol (Ceccaldi, 1952), (Neujean, 1954) or with a very small dosage are anecdotic (Robertson, 1963), (Sina, Triolo et al., 1977).

When the first series includes three daily applications followed by a 7 days interval, ES frequently occurs immediately before or at the beginning of the second series, independently of the characteristics of the dosage (increasing or constant dosage). A second peak of incidence is described in a few datasets around day 30 after the first melarsoprol application.

In a randomized trial (IMPAMEL I) comparing one group of patients (n: 250) treated with the standard Angolan National scheme (3.6 mg/kg/day, progressive increase in dosage, 3 series of 4 days with 7 days interval) with another (n: 250) receiving a new concise protocol in which a constant dose (2.2 mg/kg/body weight) of melarsoprol applied daily for ten consecutive days, Burri et al (Burri, Nkunku et al., 2000) showed that the risk of ES was the same in the two groups and that ES occurred at the same average time point independently of the scheme. Most cases occurred between day 4 and 10 for the standard schedule and between day 6 and 12 for the new scheme. In a subsequent study with 88 additional patients treated according to the new scheme the same authors obtained a maximum frequency of ES between days 6 and 10.2 (mean 10.2 days) and showed that both the coma and the convulsive types of ES tend to occur at this same time point. The mental type of ES was excluded from this analysis. In this study, additionally, ES occurred between days 22 and 26 in 5 (14.7%) out of the 34 cases (Blum, Nkunku et al., 2001). Nevertheless, the ongoing analysis of the results of IMPAMEL II (a large scale multicenter study including 7 countries, 2020 patient files, 176 ES cases) using exclusively the concise protocol does not confirm the existence of this second peak. The occurrence of ES ranged from day 1 to day 28 with a maximum between days 9 and 11 (mean 9.2, standard deviation 4 days) (Schmid, Richer et al., submitted for publication).
5.4.7 Concomitant diseases

An association between the syndrome and an influenza epidemic is described in 1957 in Abengourou, Ivory Coast. Asiatic influenza reached this region in August 1957 and in September 10 patients (5 in first stage and 5 in late stage) with newly diagnosed HAT (T. b. gambiense) and concomitantly clinically diagnosed with influenza were treated with melarsoprol. Within 5 days of treatment, all 10 patients entered a deep coma, in 9 cases with convulsions, and died within 48 hours. One post-mortem examination disclosed findings that were suggestive of advanced HAT encephalitis. Outside of the influenza epidemic, the incidence of ES in the area was in the 1% range. A morbid synergy between HAT and viral disease is postulated (Bezon and Ducasse, 1958).

In IMPAMEL II malaria was the most common concomitant infection (24.5%), followed by intestinal infection (11.2%) in 977 patients out of 2343 treated with melarsoprol. This study population showed an incidence of 41.7% of any other infectious disease diagnosed on admission or during melarsoprol treatment. 9.4% and 5.2% of the patients had 2 and 3 or more infections diagnosed. A significant association was observed between for malaria and gastrointestinal parasitosis and adverse events, including ES and death from ES (Schmid, personal communication).

5.5 Interventions used in the encephalopathic syndrome

Interventions are classified according to their objective (preventive or therapeutic), time frame (previous or concomitant to melarsoprol) and type (pharmacological or non-pharmacological). Table 10 shows the number of datasets for each intervention.
<table>
<thead>
<tr>
<th>Intervention</th>
<th>Prevention of ES</th>
<th>Management of ES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Previously to melarsoprol</td>
<td>During melarsoprol</td>
</tr>
<tr>
<td><strong>Pharmacological</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Trypanocidal drug</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Antimalarial</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Anthelminthic</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Promethazine</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Iron</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Multivitamins</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>BAL</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Mannitol, furosemide</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Neuroleptics</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Non-pharmacological</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutritional supplement</td>
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<td></td>
</tr>
<tr>
<td>Bed rest</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Transfusion</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Alcohol abstinence</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fasting</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>IV fluids</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Oxygen</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>„Intensive care“</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

Table 10: Preventive and therapeutic interventions in ES: number of citations in the reviewed literature.
5.5.1 Interventions for prevention

Interventions for the prevention of ES may take place previous or during melarsoprol administration. They were empirically designed and are mainly aimed at: limiting the potential reactions of the immune system, reducing the parasitic load (either related to the trypanosomal infection or to potentially existing concomitant parasitic diseases) and improving the patient general and nutritional status. Medications and general measures given previous to melarsoprol administration are often designated as “patient preparation”. This practice is widespread but the drugs or measures used and the duration of this “preparation” differ depending on country or region.

The most frequently cited drugs are, in descending frequency: peripheral acting trypanocidal drugs (pentamidine and suramin) and antimalarials (mainly chloroquine), corticosteroids, anthelminthics (mainly mebendazole), multivitamins, promethazine, iron, antibiotics, adrenaline, and anticonvulsants. Non-pharmacological measures are cited less often than drugs; they consist in nutritional supplementation, compulsory bed rest, alcohol abstinence, fasting before melarsoprol application and blood transfusion. Corticosteroid use, in particular, has been the subject of much debate.

Corticosteroid’s role as a preventive measure against ES is specifically addressed in 6 datasets (3 Gambiene, 3 Rhodesiense). Two are prospective controlled trials (one for each form of the disease), and one is an open study; the remaining datasets are retrospective controlled studies and in one case a combination of the above mentioned prospective controlled trial for Gambiense and a retrospective analysis. Although not designed for this purpose, the study IMPAMEL II is one additional source of information on this matter.

In the Rhodesiense studies, prophylactic corticosteroids appear to have no definite role in the prevention of ES, but are considered protective towards
less severe melarsoprol related complications and beneficial for the moribund patient, minimizing early deaths. The size of the study population is considered too small for statistical significance in one study (Foulkes, 1975); the therapeutic melarsoprol schemes are very different in another study (Arroz, 1987); and a potential confounder is the simultaneous administration of oral and parental diminazene aceturate in the third study (Onyango, Bailey et al., 1969).

In Gambiense HAT studies, the analysis of the combined studies made in the DRC by Pepin (one prospective clinical trial and two case series) suggests that prednisolone significantly reduces the incidence and the mortality of ES, especially in patients with trypanosomes observed in CSF and/or with a WBC count in CSF of 100 or more cells per mm³ (Pepin, Milord et al., 1995). In a subset of patients observed in the Sudan during the IMPAMEL II study, 202 were treated with prednisone and 449 without. 26 ES episodes were observed in the steroid group and 41 in the non-steroid (RR: 1.4; p: 0.19; CI 95%: 0.9-2.2). Incidence and CFR for ES in the two groups did not significantly differ (RR: 0.72, p: 0.26, CI 95%: 0.8-2.2 and RR: 1.4, p: 0.59, CI 95%: 0.5-3.9) (Schmid, Richer et al., submitted for publication).

The evaluation of the role of preventive corticosteroids is complicated by the different variety of drugs used: prednisone and prednisolone are used in 8 datasets; hydrocortisone, betamethasone, deltacortisone, and dexamethasone are cited in one dataset each (underpowered studies); in 3 datasets the type of corticosteroid is not stated. The often unclear and variable duration of the corticosteroid administration constitutes an additional difficulty.

The rationale for antimalarial, antihelminthic, multivitamin and iron administration, nutritional supplementation (in particular the so called “diète lactée”) and blood transfusion is based on the high prevalence of parasitic diseases, malnutrition and anemia in the population in general and in HAT patients in particular. The general opinion is that the presence of those
conditions on admission increases the patients’ susceptibility to develop adverse events when given melarsoprol. However, no comparative studies on the protective role of these interventions are available.

Melarsoprol is an extremely fast acting trypanocidal drug. Following the first melarsoprol administration fever is described as very common. This event has been associated with the massive destruction of circulating trypanosomes, especially in the Rhodesiense form of HAT, where parasitemia is usually high. The use of pentamidine (for Gambiense HAT) and suramin (for Rhodesiense HAT) was found to decrease the incidence of fever during the subsequent first doses of melarsoprol, especially in the Rhodesiense form. However, no reference to a clear role for these drugs in ES prevention was found.

A limited number of datasets report the use of promethazine, antibiotics, vitamin C, adrenaline and anticonvulsants in the prevention of ES. Administration of promethazine previous to the daily melarsoprol dose is cited in 8 datasets, but no comments on its role regarding ES were found. Penicillin is mentioned as useful in several publications, especially when a corticosteroid is administered, to protect from secondary bacterial infection and from ES, but no quantification of this protective effect is available. A comparison between the outcome of ES in two groups of patients having received prednisolone associated (n: 16) or not (n: 59) with cotrimoxazole showed an ES case fatality rate 8 times lower (6.3% versus 52.5%; RR: 0.12; CI 95%: 0.02-0.81%) in the antibiotic group. In this same study the concomitant administration of melarsoprol and thiabendazol or praziquantel was considered a risk factor for ES (Ancelle, Barret et al., 1994). No additional reference to the role of systemic diffusible antelminthic drugs in ES was found. Vitamin C in large doses has been tried in an underpowered trial and results were not convincing (Buyst, 1975). An open, partially controlled trial showed that adrenaline has no role in the prevention of ES (Sina, Triolo et al., 1982).
As for non-pharmacological measures, mandatory bed rest is part of several protocols, as ES has been observed in discharged patients that perform long extenuating walks going home. Mandatory alcohol abstinence during melarsoprol treatment is mentioned only twice but alcohol consumption (between melarsoprol series or after patient discharge) is cited as correlated to ES.

5.5.2 Interventions for treatment

Therapeutic interventions in ES are, in descending order of frequency, those aimed at: neutralizing the hypothetical arsenical excess, limiting the hypothetical immunological phenomena involved, managing the manifestations of CNS damage and raised intracranial pressure (ICP) and correcting metabolic disturbances.

BAL (British anti-lewisite: dimercaprol, a heavy metal chelator) was used in many therapeutic protocols for ES as an antidote for melarsoprol. The use of BAL was largely abandoned following the analysis of 63 ES cases observed in the DRC suggesting that BAL administration was associated with a higher ES mortality (CFR with BAL 86% versus 57% without BAL, p: 0.047, OR: 4.5, CI: 1.05 -26.87) (Pepin, Milord et al., 1995).

The precise role of corticosteroids in the treatment of ES is difficult to access. Although corticosteroids are said to have a beneficial role in reducing case fatality rate, numbers are not available. The type of corticosteroid and dosage are also seldom clearly stated. In 7 datasets the type of corticosteroid is not specified, in 3 others hydrocortisone is used and in one dexamethasone is applied.

Adrenaline was found to be useful in the management of ES (7 out of 10 ES patients receiving adrenaline survived). Hypertension and the need for frequent administration were cited as the two main drawbacks of this drug (Sina, Triolo et al., 1982). Although adrenaline is recommended in several publications for management of ES its use is not frequently mentioned.
Management of convulsions usually includes the use of phenobarbital (8 datasets) or diazepam (4 datasets). Regarding the value of anticerebral edema drugs in ES, the information is very scarce. The most frequently used drug is mannitol, sometimes combined by furosemide. The use of furosemide alone is anecdotic (2 patients). In this same dataset a reference is made to a cisternal tap performed in an ES patient with an excellent result (Onyango and Ogada, 1968).

The number of datasets where measures aimed at general support and metabolic imbalance correction are cited is low (see table 10). These measures include intravenous fluid administration in 6 datasets, oxygen in 1 dataset and a combination of general measures for monitorization and correction of metabolic abnormalities (“intensive care”) in 5 datasets. The number of ES patients receiving a “high” level of care is too low to allow conclusions on the impact of those measures on ES case fatality rate.

We performed a logistic regression using the identified pharmacological and non-pharmacological data concerning interventions for prevention and treatment of ES. Data was extractable from 29 datasets for preventive interventions, from 15 datasets for during melarsoprol interventions and from 25 datasets for the characteristics of ES treatment. For preventive and during melarsoprol interventions the used binary outcome was the existence or not of an ES; for therapeutic interventions the binary outcome was death or survival from an ES. We expanded the numbers in each study in MS Excel® to compensate for random size effect. The analysis was carried out using Stata 7.0. Results for the role of corticosteroids and peripheral acting trypanocidal drugs are shown in table 11. No single intervention or combination of interventions was found to have a protective effect in the prevention or outcome of ES.
### Table 11: Logistic regression showing data for corticosteroid and peripheral acting trypanocidal drugs

<table>
<thead>
<tr>
<th>Interventions</th>
<th>Odds Ratio</th>
<th>Standard error</th>
<th>z</th>
<th>P &gt; Izl</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple (bivariate) regression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral trypanocidal drug</td>
<td>2.18</td>
<td>0.22</td>
<td>7.66</td>
<td>&lt; 0.0001</td>
<td>1.78 - 2.66</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>1.89</td>
<td>0.18</td>
<td>6.62</td>
<td>&lt; 0.0001</td>
<td>1.57 - 2.29</td>
</tr>
<tr>
<td>Adjusted (multivariate) regression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral trypanocidal drug</td>
<td>1.87</td>
<td>0.20</td>
<td>5.80</td>
<td>&lt; 0.0001</td>
<td>1.51 - 2.31</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>1.52</td>
<td>0.15</td>
<td>4.12</td>
<td>&lt; 0.0001</td>
<td>1.24 - 1.86</td>
</tr>
</tbody>
</table>

5.6 **Consequences of the Encephalopathic Syndrome**

5.6.1 **Outcome and sequelae**

ES is usually described as a catastrophic event. In the reviewed literature it is described as appearing rapidly and unexpectedly (minutes or a few hours after the last melarsoprol application), and usually resolving within 24 to 48 hours, especially for the forms with convulsions or coma. Longer durations before the final outcome are described, with patients surviving up to 10 days after an ES event. The coma form is associated with a higher mortality. The mental form has a more prolonged resolution and is more benign, although the psychotic manifestations are difficult to control.

The most often cited result of ES in the reviewed literature is complete recovery without neurological or other sequelae.

5.6.2 **Resumption of melarsoprol after an encephalopathic syndrome**

In the reviewed literature we found a total of 42 patients (from 4 datasets for Gambiensen and from 3 datasets for Rhodesiense) to whom melarsoprol was given again after an ES event without any additional complication. These patients give evidence that ES does not recur when melarsoprol treatment is resumed.
5.7 **OTHER ADVERSE EVENTS**

The exact prevalence of melarsoprol-associated adverse events different from ES is difficult to access in HAT. Dermatitis (including bullous, exfoliative dermatitis and Lyell syndrome), diarrhea (including the so-called aparasitary arsenical diarrhea) and fever constitute the more frequently cited adverse events. The number of datasets referring to adverse events and the type of adverse event is shown by order of descending frequency in table 12.

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Number of datasets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatitis</td>
<td>14</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>10</td>
</tr>
<tr>
<td>Fever</td>
<td>7</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>3</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>3</td>
</tr>
<tr>
<td>Albuminuria</td>
<td>2</td>
</tr>
<tr>
<td>Collapse</td>
<td>2</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2</td>
</tr>
<tr>
<td>Angioneurotic edema</td>
<td>1</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>1</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>1</td>
</tr>
<tr>
<td>Necrosis</td>
<td>1</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>1</td>
</tr>
<tr>
<td>Hematuria</td>
<td>1</td>
</tr>
<tr>
<td>Jaundice</td>
<td>1</td>
</tr>
<tr>
<td>Hepatic failure</td>
<td>1</td>
</tr>
<tr>
<td>Pruritus</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 12: Adverse events cited in 22 datasets: 14 referring to the Gambiense (4343 patients) and 8 referring to the Rhodesiense form (1070 patients).
6 DISCUSSION

6.1 METHODOLOGY USED

The Cochrane Group methodology was useful in clearly establishing the objectives of this study, in defining the outcomes to measure, in the design of the systematic search for relevant datasets, and in determining the choice of parameters to be critically appraised. Due to the very heterogeneous and frequently poor quality of the reviewed literature the methodology was, however, difficult to apply for the analysis and synthesis of extracted data.

In order to maximize the chances of collecting useful data and given the observed characteristics of clinical studies in HAT and in ES, we used an open criterion for the electronic search methodology. The low sensitivity of this strategy is reflected in the considerable number (2500) of references that had to be scanned for relevance as compared to the final number of included datasets (157). The specificity of our subject was addressed with more precision by searching the selected 3 experts’ literature databases, although only one of these was fully electronically searchable. This is linked to the fact that many important references in HAT are published in old, difficult to find or non-indexed journals. In this context, using reference lists for “citation chasing” was particularly productive. A duplication bias (the same set of patients described in more than one study) was also found in a significant number of datasets (11). Our initial expectation of finding valuable data in unpublished papers and grey literature was not fulfilled: generally, important findings were published.

In the relevant datasets referring to clinical studies, data extraction was complicated mainly by imprecision in the description of the characteristics of the study population, the staging of HAT, the treatment schedules, and the definition of ES. Precision was also missing in the description of the methodology used and in the presentation of the results. Additionally, the reviewed literature covers a time frame of half a century, and includes clinical
data from many different historical, geographical, cultural and scientific backgrounds.

Although individual case descriptions frequently possess high quality, research was clearly not the main priority for the medical doctors having to deal with huge numbers of sleeping sickness patients. This accounts for the mainly retrospective nature of the datasets. To address each specific issue we had to select the datasets where the main measured outcomes (existence of ES and ES case fatality rate) where also available; this invariably caused an additional reduction in the number of datasets available for analysis and consequent loss of power.

All the described limitations in data quality and potential sources of bias hindered the acquisition of solid and robust evidence and of clear answers to the issues addressed.

6.2 Epidemiology of the Encephalopathic Syndrome

Not surprisingly, studies on the Gambiense form of HAT, which is associated with a greater burden of disease, yield nearly 82% of all described melarsoprol treatment courses and 72.5% of all the ES cases. On the other hand, Rhodesiense yields a higher incidence risk for ES. Explanations for this should include the fact that Rhodesiense is a more acute and aggressive disease than Gambiense. In *T. b. rhodesiense* infection parasitemia is higher and patients are frequently diagnosed in a more advanced stage and show more complications (hence the higher overall mortality for Rhodesiense).

Another possible reason could be related to the characteristics and quality of the datasets regarding the diagnosis of ES. The incidence risk for ES clearly varies from country to country but, more significantly, is also very different from study to study in the same country. A “good” example of this type of bias comes from the two datasets describing ES cases in Kenya: they establish the minimum (1.5%) and the maximum (28%) incidence risk for ES in Rhodesiense. A linear regression trend line for the number of patients treated
shows an imperfect negative correlation ($R^2$: 0.3262) between the number of patients treated with melarsoprol and the incidence risk of ES.

ES case fatality rate also varies widely from country to country and for each study within a same country, but the influence of the two forms of HAT is not obvious for this indicator: maximum, minimum and median values are similar in both forms. A possible bias in the case of ES case fatality rate is the attribution of the cause of death, since in advanced cases it is difficult to discriminate between the manifestations of ES and those of HAT itself.

6.3 Definition of the encephalopathic syndrome

How is ES defined? In the absence of biological markers for the phenomenon, the answer to this question should come from the analysis of clinical data and from pathology studies. Unfortunately, the number of anatomopathological studies on ES is very limited.

In order to try to obtain the least possible biased definition of ES, we analyzed the characteristics of ES in datasets that used a precise pre-established definition of the phenomenon (i.e. patient conscious, not convulsing and without psychotic changes on admission). We subsequently selected datasets simultaneously meeting these criteria and including at least 4 cases of ES. The selected datasets describe a total number of 221 patients with ES. In 40% of them the pattern of ES consisted in convulsions followed by coma. In the remaining datasets ES consisted in overlapping neurological (convulsions, coma) and mental (sometimes psychotic) manifestations. The frequency of the mental manifestations was lower than the neurological ones and they could precede or accompany convulsions or coma. In smaller case series, case reports and in datasets where the quality of the definition criteria for ES is less precise, the same variability in the clinical manifestations of ES was observed, despite a comparatively smaller number of observations. ES consisting exclusively of coma, or convulsions or mental manifestations was also cited less frequently. In fact, a reference to a clear
separation between the 3 manifestations of ES was found in only two publications.

Since the number of datasets that indicate a mixed type of ES manifestations is comparatively higher, we might contemplate that ES consisting in convulsions or coma isolated may constitute an observational bias. Given the generally low quality of medical records available in the rural medical facilities where HAT patients are managed and the retrospective nature of the majority of studies, an initial single episode of convulsion of short duration preceding coma could quite easily go unreported. Conversely, a post-ictal phase might be wrongly interpreted as coma and the im temperate administration of anticonvulsants could help precipitate a decrease in the patient’s level of consciousness.

Haller introduced a distinction between three distinct types of ES based on clinical data from 14 patients with ES and on CNS postmortem findings from 5 of them. Haller described: 1) an acute convulsive status, with elevated intracranial pressure, papilloedema and a high albumine content of the CSF, corresponding to severe hypoxic brain damage in 2 cases, associated with a fatal outcome in 3 of the seven cases 2) a rapidly developing coma without convulsions associated with an acute hemorrhagic leukoencephalitis in 2 cases (Figure 9), one with concomitant hypoxic brain damage, associated with a fatal outcome in 2 of the 3 cases and 3) mental disturbance, mainly aggressivity, restlessness or depression without other neurological signs, associated with survival in all 4 cases. Three of the 5 patients how died had first stage HAT. The hemorrhagic leukoencephalitis included severe abnormalities in the brain stem, with fibrinoid necrosis of small vessels and ring and ball hemorrhages. Sparing of the Purkinje cells in the cerebellum was considered suggestive of hypoglycemia. One autopsy revealed acute purulent meningitis, emphasizing that clinically undiagnosed CNS conditions may constitute a confounder in the determination of the patient’s cause of death (Haller, Adams et al., 1986) (Adams, Haller et al., 1986).
Two autopsies of patients in Rhodesiense first stage HAT treated with melarsoprol and developing a coma of sudden onset without convulsions are also available (Manuelidis E.E, Robertson D.H et al., 1965). Hemorrhages were macroscopically detected in the midbrain, pons and caudate nucleus; histology showed extensive bleeding in the midbrain, thalamus, tegmentum, pes pedunculi and in the cerebellar white matter in one case and in the midbrain pons, basal ganglia and caudate and lentiform nuclei in the other. Evidence of meningitis or perivascular cuffing suggestive of HAT were absent in both cases.

We searched for anatomopathological similarities between ES and other encephalopathic conditions. Similarities were found between ES and acute hemorrhagic leukoencephalitis, (AHLE), a condition also called “Hurst disease” or “acute Hurst hemorrhagic encephalitis”. This denomination is presently attributed to a very rare form of acute hemorrhagic and necrotic leukoencephalitis which is tough to represent the severe and usually fatal variant of acute disseminated encephalomyelitis (ADEM), an uncommon inflammatory demyelinating condition of the CNS. ADEM is considered by
some authors as part of the clinical spectrum of multiple sclerosis (Garg, 2003).

AHLE is observed in all age groups, but predominantly in adolescents and young adults, and is often preceded by a respiratory disease (Donnet, Dufour et al., 1996), (Voudris, Skaardoutsou et al., 2001), (Pfausler, Engelhardt et al., 2002), (Garg, 2003). The etiology of the phenomenon is obscure and an immunological basis is hypothesized. Using susceptible animals, the pathological findings observed in ADEM and AHLE can be reproduced in experimental allergic encephalomyelitis (EAE) and in hyperacute experimental allergic encephalomyelitis (HEAE), respectively. Evidence obtained from EAE and HEAE suggests the implication of an autoimmune response against myelin or other autoantigens. This response is thought to be mediated by T-cells (alone or in synergy with antibodies) that recognize structurally myelin peptides similar to microbial epitopes and launch autoaggressive attack on CNS structures (“molecular mimicry”) or via a non-specific activation of an autoreactive T-cell clone (Scully, Mark et al., 1995). The anatomopathological characteristics of brain damage in AHLE consist in necrotising vasculitis of venules, multifocal and diffuse perivascular petechial, ring and ball hemorrhages and infiltrates (Murthy, 2002). These anatomopathological changes are very similar to the ones described in the coma type of ES. Patients with Hurst disease usually develop an acute CNS condition but, like in ES, different clinical expressions exist. (Huang, Chu et al., 1988), (Yoshikawa, Watanabe et al., 1999)

The clinical expression of a particular type of encephalomyelitis variant is possibly determined by a peculiar combination of individual genetic susceptibility and a given type of triggering infectious challenge (Scully, Mark et al., 1999). In a recent study on 38 Russian patients, ADEM was found to be associated with the Major Histocompatibility Complex (MHC) class II antigens DRB1*01 and DRB1*017(03) and preferential clinical patterns were
found to be associated with specific viral triggers (Idrissova, Boldyreva et al., 2003).

No controlled clinical trials exist to guide the therapy of ADEM or AHLE. Successful management of the conditions has been described with massive doses of corticosteroids (de Crouzas, Deruaz et al., 1975), (Straub, Chofflon et al., 1997), (Meilof, Hijdra et al., 2001). When corticosteroid therapy fails, plasma exchange, cyclophosphamide and IV immunoglobulin have been shown to be helpful (Kanter, Horensky et al., 1995), (Schwarz, Mohr et al., 2001), (Marchioni, Marinou-Aktipi et al., 2002). Decompressive craniotomy with dural augmentation has been recently described in two patients with Hurst disease that failed to respond to the best possible medical therapy for increased intracranial pressure, with good immediate and long term results (Taferner, Pfauzl et al., 2001). Early initiation of therapy is important for prognosis but patients in deep coma may fully recover. Although there are no clear-cut in vivo diagnostic criteria for AHLE, MRI is the best alternative for differential diagnosis, together with lumbar puncture (Coyle, 2000).

A phenomenon similar to ES also occurs outside the HAT context and with drugs other than melarsoprol.

Although inorganic arsenicals were known and used in human medicine since ancient times, organic arsenicals were introduced mainly, but not exclusively, for the treatment of syphilis at the end of the beginning of the 20th century, in substitution of the more toxic and inefficient mercurial, bismuth, sulphur and iodure traditional preparations (Martinet, 1926).

The first description of “hemorrhagic encephalitis” associated with their use in syphilis was published in 1903. The phenomenon was latter termed “arsenical encephalopathy”. A total number of 83 published cases of “arsenical encephalopathy” in which the diagnosis was confirmed by necropsy were found in a review published in 1949 (Call and Gunn, 1949). The reported incidence risk for the reaction was 1 in every 63'000 organic arsenic
injections (Stokes, Beerman et al., 1941) and case fatality rate corresponded to one death in every 5398 patients treated and 1 in every 28758 injections (Glaser, cited by Call 1949).

The phenomenon usually appears 48 hours after the third injection of an organic arsenical and consists, in order of frequency, of: headache, confusion, irritability, followed by nausea, vomiting, stupor, convulsions, hyperpyrexia and death. Additional neurological manifestations include: changes in deep reflexes, loss of corneal reflex, disturbances of speech, loss of light reflex, tremors, hemiplegia, nystagmus, and facial palsy. Recoveries were reported but case fatality rate was in the 50-75% range. Neuropathological studies showing varying degrees of severity of the same essential process in the CNS were classically described by Wilson (Wilson, 1940): “abundant small perivascular effusions, mostly of the ring type; plugged capillaries with minute necrotic zones around them (...) interstitial reactions otherwise are never pronounced and frequently lacking (...) at times however nothing more than congestion and edema is discovered (...) moderate demyelination around hemorrhagic zones is also seen occasionally”. Predilection was for the white substance of the brain and for certain zones like the corpus callosum, the caudate and lenticular nuclei the pons and medulla, although all the brain substance could be involved. These varying degrees of severity were thought to correspond to the different observed clinical syndromes. Elemental arsenic levels were generally reported as “unusually high” in CNS tissue (Call and Gunn, 1949). The pathogenesis of arsenical encephalopathy was never satisfactorily determined. The commonly accepted theory involved vascular damage, either associated with a direct toxic action of the arsenic preparations or with a delayed type of immunological reaction.

In a small open trial melarsoprol was used to treat 8 patients refractory to multiple chemotherapy regimens for leukemia. Melarsoprol was given in escalating daily doses in 3 series. Three patients experienced generalized convulsions during their second week of therapy. One of the patients had
sub-therapeutic levels of phenytoin, and after correction convulsions ceased. The second patient had one single convulsive episode and recovered without sequelae. The third patient died from fever and coagulopathy. Melarsoprol and elemental arsenical levels were measured in blood and urine (but not in CSF) using respectively High Performance Liquid Cromatography (HPLC) and induction-coupled plasma-mass spectroscopy methods. No difference in peak plasma concentrations during the first cycle of treatment was observed between patients developing or not convulsions. A gradual increase in plasma levels of elemental arsenic was detected over the 3-week treatment course. A direct toxic effect of melarsoprol on the CNS was postulated (Soignet, Tong et al., 1999).

ES, or a syndrome considered similar to ES, is also described in loiasis treated with albendazol (Blum, Wiestre et al., 2001), ivermectine (Gardon, Gardon-Wendel et al., 1997), (Boussinesq, Gardon et al., 1998) and diethylcarbamazine (Carme, Boulesteix et al., 1991); and in onchocercosis treated with mel W (Duke, 1966).

ES is observed in HAT patients treated with the non-arsenical drugs antrypol, diminazene aceturate (de Raadt, 1966), and suramin (Burri and Blum, 1996); with other arsenicals like tryparsamide (Mackie, 1935), melarsen (Butler, Duggan et al., 1957) and mel W (Collomb, Zwingelstein et al., 1963), (Sow, 1965). In addition, ES is also described in 3 melarsoprol relapsing patients treated with eflornithine (Pepin and Milord, 1991).

Except for the well documented acute hemorrhagic leukoencephalitis and “arsenical encephalopathy” studies, the reported number of patients suffering from these ES-like conditions is very limited and no pathology studies are available.

It remains thus to be demonstrated that 3 different clinical types of ES exist, corresponding to different anatomopathological aspects and etiologies, in opposition to different presentations within the spectrum of the same
phenomenon. Clinical and pathological analogies between ES and “arsenical encephalopathy” and especially with AHLE and ADEM suggest that the second hypothesis should be favoured and that the different clinical presentations of ES may be determined by the intensity of the primary pathological immune reaction. Definitive elucidation of the existence of common or separate types of ES calls for correctly designed clinical studies and will ultimately rely in neuroanatomical evidence.

6.4 **Etiology of the encephalopathic syndrome**

What is the cause of ES? Efforts in the determination of the nature and pathogenesis of ES are hampered by the ethical and logistic limitations in performing human experimentation on the phenomenon. Animal models and *in vitro* methods were designed to try to overcome these limitations.

Both the acute and chronic stages of HAT can be reproduced in several animal models: sheep (Bouteille, Darde et al., 1988), mice (Bouteille, Darde et al., 1987), (Jennings, McNeil et al., 1989), rats (Schultzberg, Ambatsis et al., 1988), (Anthoons, Van Marck et al., 1989) and monkeys (Schmidt, 1983) (Bouteille, Millet et al., 1998) can be infected with specific *T. brucei* species. They offer the possibility of investigating the pathogenesis of HAT, and of testing drug activity and pharmacokinetics. The different animal models differently reproduce aspects of human disease.

In the murine model that best reproduces ES, exposure to trypanocidal drugs that do not significantly cross the blood brain barrier or to sub-curative levels of melarsoprol results in the development of CNS pathological changes that are similar to human ES: the so called post-treatment reactive encephalopathy (PTRE) (Hunter, Jennings et al., 1992). In this model the main working hypothesis for the etiology of ES is that it is the response against trypanosomes persisting in the CNS, via activated astrocytes, that triggers PTRE (Hunter and Kennedy, 1992). The anti-inflammatory and immunosuppressive drug azathioprine abrogates the development of PTRE,
although the phenomenon was observed approximately 15 days after cessation of the drug (Hunter, Jennings et al., 1992). Exposure to high levels of melarsoprol also inhibits PTRE (Jennings, Hunter et al., 1993). A topical formulation of melarsoprol may be used in this model to obtain sustained curative levels of the drug, also leading to resolution of PTRE (Atouguia, Jennings et al., 1995). This sub-curative dose theory for ES was seriously challenged by the findings of the IMPAMEL I study, where the incidence and time point for the occurrence of ES during a constant drug pressure corresponding to the application of a daily dose of 2.2 mg/kg of melarsoprol for 10 days were found to be similar to the ones observed with any other melarsoprol scheme (Burri, Nkunku et al., 2000).

The anatomopathological findings in human ES are very similar in topographic localization and histological damage to the ones induced by Hurst in monkeys (and partially in rabbits) using tryparsamide and three other trivalent arsenical compounds (phenylarsine oxide, arsenious oxide and novaarsenobenzene). The author mentions that his findings in the animal model are similar to the arsenical encephalopathy found in man from the early days of therapy with organic arsenicals. Relative over-dosage with abnormally high levels of the arsenical due to a metabolic individual characteristic was considered a plausible pathogenetical hypothesis (Hurst, 1959).

The discussion of the above cited papers indicates that the clinical features of ES appear to be similar to the ones observed in “arsenical encephalopathy” and in AHLE. These features include the unpredictability of the phenomenon, the suddenness in installation, the variability of the manifestations, the lack of biological markers and the bad prognosis. The neuroanatomical characteristics of ES also bear many similarities in terms of type of histological damage and topographical localization with the ones observed in “arsenical encephalopathy” and in AHLE.
These analogies support the concept that in these three conditions a common delayed hypersensitivity-like immunopathogenic process (irrespective of the triggering and precipitating factors) exists in which the endothelium of brain capillaries and/or microglia cells in brain parenchyma are mistargeted for immune attack, resulting in loss of endothelial integrity and perivascular effusions in certain susceptible areas of the CNS. The brain parenchyma responds to this insult with a pathological reaction (which is common to many physical, chemical or biological CNS provocative agents) mediated in part by the release of immune mediators including cytokines, prostanoid products and nitric oxide (NO) (Rivest, Lacroix et al., 2000), that results in congestion, edema, interstitial infiltration and, if the process is severe enough, in demyelination and necrosis.

What are the distinctive characteristics of ES? The main overall difference is the incidence, which is much higher in melarsoprol associated ES than in “arsenical encephalopathy” or in AHLE.

A specific difference between ES and the other encephalopathies must be linked to the presence of the infecting trypanosome in body compartments, especially in the CNS. Humans respond to trypanosomal infection with changes in the production of TNF-α, INF-γ, IL-1β, IL-6, IL-10, PGE (Askonas, 1984), (Rhind, Sabiston et al., 1997), (Lejon, Lardon et al., 2002) and NO (MacLean, Odiit et al., 2001). Body compartments (blood, CNS) react differently and are differently affected by these changes. The combined effects of systemic and CNS cytokine imbalance result in deleterious changes in the blood brain barrier permeability and CNS immune function, including the upregulation of existing clones of autoreactive cells. Antibodies directed to myelin (Kobayakawa, Louis et al., 1979), (Hunter, Jennings et al., 1992), galactocerebrosides (Girard, Bisser et al., 2000) or neurofilaments (Ayed, Brindel et al., 1997) have been demonstrated in HAT. Furthermore, a recent study showed that CSF from late stage HAT patients induce apoptosis in microglia and endothelial cells and contain soluble Fas Ligand (sFasL) and
anti-Fas antibodies, which are potent inducers of the cell death receptor Fas (CD95) (Girard, Bisser et al., 2003). A recently identified trypanosomal apoptotic factor (TAF) was found to mediate human brain vascular endothelial cells apoptosis. TAF may be a procyclin or procyclin derivative (Stiles, Whittaker et al., 2004). Melarsoprol in sufficient levels would then act as a trigger for a specific immune reaction (cellular, humoral or a combination of both), producing intensification of the signaling for programmed cell death and resulting in extensive CNS damage by means of an uncontrollable positive feedback loop in immune cell and cytokine network.

Why is this hypothetical unfortunate combination of a peculiar host immune response to trypanosomal infection and susceptibility to melarsoprol expressed only in selected patients?

Data on the biotransformation of melarsoprol in humans is severely lacking. Preliminary studies in HAT patients showed that the route of excretion is mainly biliar (Cristeau, Placidi et al., 1975). In the rat model melarsoprol was also found to be eliminated by hepatobiliary transport. This transport is partially glutathione (GHS) dependent, as GHS depletion diminished but did not abolish the biliary output of the arsenical. The drug induces a partial depletion of hepatic GHS. HPLC analysis revealed the presence of a major unidentified metabolite, most likely consisting in a melarsen-glutathione conjugate. Two other melarsoprol metabolites were also revealed, identified as melarsoprol-glucoronide conjugates (Gregus and Gyurasics, 2000). In an independent work, melarsen was found to be covalently bound to serum proteins in humans (Keiser, Ericsson et al., 2000). GSH conjugation and glucoronidation are thus considered partially complementary processes in the elimination of melarsoprol. These findings, although requiring confirmation in humans, might indicate that GHS is needed for elimination and detoxification of melarsoprol and that GHS might be depleted from tissues during this process (Gregus and Gyurasics, 2000). Protection mechanisms
against damage caused by oxidants and electrophiles, including the arsenicals themselves, are frequently GHS-dependent (Hayes and Strange, 2000).

Pharmacological data is available from a limited number of samples from HAT patients treated with melarsoprol. Melarsoprol pharmacokinetic studies using both a biological assay and electro thermal atomic absorption spectroscopy showed remarkable interindividual variability in the drug levels in CSF after each course and at the end of treatment. Furthermore, drug levels measured in the CSF were generally very low (about 50 times lower than those in serum), never exceeding 10% of the serum levels (Burri, Baltz et al., 1993). In uninfected vervet monkeys the interindividual variability in melarsoprol CSF levels measured using the same bioassay was markedly smaller than the one observed in human patients with HAT. In some single human samples CSF drug levels were two-fold higher than in monkeys. These findings seem to indicate that a variable degree of blood brain barrier inflammation exists in each patient and that this variability influences melarsoprol penetration into the CNS compartment (Burri, Onyango et al., 1994).

A comparative preliminary analysis of the population pharmacokinetics of melarsoprol in patients developing or not developing ES during IMPAMEL I showed a slight prolongation in melarsoprol half-life in patients developing ES but the sample size does not allow drawing firm conclusions (Burri, unpublished data). An extremely high content of urinary total arsenic is described in a single HAT (T. b. rhodesiense) patient with a clinical diagnosis of ES after the second course of melarsoprol. In this study, which enrolled 28 patients treated with melarsoprol, no correlation was found between estimated creatinine clearance and urine arsenic values (Harrison, Harris et al., 1997).
To the best of our knowledge, additional studies measuring melarsoprol, melarsoprol metabolites or elemental arsenic levels in the CSF or in the CNS during or after ES are missing.

Although recognizing that ES is likely an immunological phenomenon, one can not completely dismiss the hypothesis that ES is triggered by abnormally high levels of melarsoprol or melarsoprol metabolites in the CNS of metabolically predisposed patients.

Individual susceptibility for the development of ES is thus hypothetically associated with a combination of (at least) two host-related factors: the type and intensity of individual response to parasite-induced disruption in immune cell function and cytokine network particularly in the CNS, including the intensity of damage to the blood brain barrier and consequent increased drug permeability; and individual characteristics in melarsoprol metabolism leading to unusual high levels of the drug in the CNS.

Both factors are at least partially genetically determined: the first is possibly related to genes controlling the immune response to trypanosomal challenge (by analogy with ADEM, genes associated with the MHC appear as potentially strong candidates) and the second by genes controlling drug metabolism. In humans, cytochrome P450-dependent reactions are responsible for an important percentage of phase I (increase in substrate hydrophilicity by introduction a reactive center into the substrate molecule) and phase II (conjugation with endogenous compounds such as glucoronic acid, sulphate, glutathione, glycine) biotransformation of endogeneous and exogenous compounds (Tamasi, Vereczkey et al., 2003). The brain holds a small percentage (approximately 0.5 to 3%) of the liver contents in cytochrome P450 enzymes, in highly localized discrete areas. Although brain P450 enzymes do not significantly contribute to overall drug transformation, the local CNS action or concentration of neuroactive drugs may be altered (Nicholson and Renton, 2002).
Most cytochrome P450 genes and regulatory factors are genetically polymorphic. Individual dosage requirements for several commonly used drugs may vary more than 20-fold depending on the different genotypes or isoenzyme expression status and may cause abolished, quantitatively or qualitatively altered or enhanced metabolism. Lack of drug efficacy and drug toxicity may be associated with the presence of multiple active gene copies and with detrimental mutations and defective alleles (Ingelman-Sundberg, 2002).

Exogenous factors can influence the expression of genetic traits. The role of infectious diseases as one of the main driving forces for human MHC polymorphism is presently well established (Damian, 1997). In animal and in vitro models of chronic trypanosomal infection evidence exists that the presence of trypanosomes is capable of modifying the MHC Class I and II antigen expression, including in the CNS (Schultzberg, Olsson et al., 1989), (Bakhiet, Olsson et al., 1990), (Pentreath, Cookson et al., 1994), (Sacco, Hagen et al., 1994), (Namangala, Brys et al., 2000). On the other hand, it has been shown that some brain cytochrome P450 forms are downregulated during localized inflammatory responses in the brain (Renton, 2000). This effect is accompanied by a similar loss of cytochrome P450 in the liver (Shimamoto, Tasaki et al., 1999), (Renton and Nicholson, 2000). Increased ability for survival in the CNS of certain strains of trypanosomes in sites not easily reached by therapeutic drugs has been hypothesized (i.e. choroid plexus (Engelhardt, Wolburg-Buchholz et al., 2001)). Additional hypothetical exogenous factors involved in ES should also include: the role of a second triggering concomitant infection with neurotrophic infectious agents, pre-existing sub-clinical and undetected arsenic levels from ambient source (Atadzhanov, 2002) and nutritional micronutrient imbalance (Golden, 1992).

The characteristics of the interaction between these endogenous and exogenous factors should determine the appearance and the intensity of the reaction leading to ES as well as the clinical presentation of the phenomenon.
6.5 Diagnosis and Differential Diagnosis of the Encephalopathic Syndrome

A diagnosis of ES should be established in a HAT patient undergoing or having finished melarsoprol therapy in the previous 30 days and developing de novo convulsions, coma, psychotic changes or a combination of these manifestations. Although ES is frequently described to appear “like a bolt in the blue”, the development (or sudden intensification) of fever, headache, nausea, vomiting, dizziness, tremors and possibly conjunctival hyperemia are the most common heralding signs preceding the phenomenon.

ES diagnosis becomes less clear if the patient had, previous to melarsoprol administration, a preexistent decreased level of consciousness, convulsions or psychotic manifestations. In this case, ES can only be suspected and the decision of prompting ES therapeutic protocols has to be taken on an individual basis.

As for other CNS conditions, diagnosing increased intracranial pressure (ICP) during ES should be important for patient management to avoid further CNS damage. In the context of ES, increased ICP may be suspected in a rapidly deteriorating comatose or convulsing patient when a combined change in pulse rate, hyperventilation and hypertension develops. The development of sudden intense headache and unexpected and projectile vomiting are also strongly suggestive of increased ICP. The optic fundi examination is helpful as it may disclose papilledema. Computerized tomographic (CT) scans are most helpful in establishing the diagnosis of increased ICP. Since CT scans are seldom available in the context where ES occurs, the clinician will have to rely solely on clinical skills to diagnose increased ICP.

In the absence of a diagnostic test for ES it seems worth to perform tests aimed at excluding conditions that are potentially associated with the development of convulsions and/or coma in a HAT patient undergoing melarsoprol treatment. These should include diagnostic tests for viral, bacterial and other parasitic diseases affecting the CNS in tropical Africa.
Lumbar puncture is required for the differential diagnosis. Performing a lumbar puncture in a convulsing patient and/or with existent evidence of raised ICP is of course far from being an innocuous procedure and should only be undertaken by skilled hands. The benefit lies however in the possibility of diagnosing conditions that are potentially treatable. Many CNS infectious conditions may potentially mimic ES in a patient in the neurologic stage of HAT. The list of the more relevant conditions includes bacterial meningitis, CNS tuberculosis, cryptococcosis, cysticercosis, syphilis, and toxoplasmosis as well as cytomegalovirus, herpes simplex and Human Immunodeficiency Virus (HIV) encephalitis. Their diagnosis requires adequate facilities to perform appropriate serologic tests, cultural or PCR techniques and CNS imaging techniques that are very rarely found in the medical context where ES is observed, meaning also that their true incidence in tropical Africa is defectively known (Atadzhanov, 2002).

The strongyloidiasis hyperinfection syndrome (SHS) should also enter the differential diagnosis of ES. SHS is defined by infestation of *S. stercoralis* in organs not normally involved in the helminthic cycle such as the liver, spleen, genitourinary tract and CNS, often leading to overwhelming and disseminated infection. SHS is almost always related to corticosteroid usage (Genta, 1992). In cases involving the CNS, *S. stercoralis* larvae may be found obstructing brain capillaries, producing micro-infarctions. Mortality of the disseminated form may reach 70% and is frequently associated with secondary gram-negative bacteremia and meningitis. Diagnosis may be difficult to establish, frequently requiring multiple stool samples or stool cultures before larvae are identified. Blood eosinophilia is however a frequent feature and in an adequate epidemiological context (like the one where HAT patients are managed) should rise the suspicion index for strongyloidiasis (reviewed by Schaeffer, Buell et al., 2004). Blood eosinophilia should also prompt diagnostic procedures for the exceedingly rare CNS forms of *Schistosoma mansoni* infestation.
In hyper and meso-endemic malaria areas the interpretation of a positive Plasmodium smear is difficult, as parasitemia does not necessarily mean that malaria is responsible for the patient clinical manifestations. The issue is further complicated if the patient received antimalarials in the “preparation” period. On the other hand, a positive Plasmodium smear in a critical ES patient can not easily be dismissed.

Circumstantial and experimental evidence indicates that additional methods, available in more sophisticated medical contexts, may have a place in the diagnosis of ES.

Magnetic Resonance Imaging (MRI) has been used in a sleeping sickness patient in France to differentiate between HAT encephalitis and ES. MRI of the brain effectively excluded (in the opinion of the authors, based in the absence of brain edema or hemorrhagic lesions) ES and melarsoprol was continued, leading to the complete resolution of the patient severe HAT encephalitis (Sabbah, Brosset et al., 1997).

In Ivory Coast, electroencephalography (EEG) was used to monitor melarsoprol and eflornithine therapy. Unlike two other melarsoprol treated patients, one individual showed no improvement of the EEG recording while under treatment. The authors speculate that this should be interpreted as a sign of refractoriness to melarsoprol and of the possible development of encephalopathy, possibly due to the drug (Hamon and Camara, 1991).

There is increasing evidence that polysomnographic recordings (consisting in a continuous recording of the electroencephalogram, electromyogram and electro-oculogram) in sleeping sickness patients allow the precise identification of alterations in the sleep/wake cycle that correlate to the severity of clinical symptoms and laboratory abnormalities and to the intensity of CNS damage in HAT. The abnormalities vanish progressively when drug therapy is successful (Buguet, Tapie et al., 1999), (Buguet, Bourdon et al., 2001). The method has been perfected and is now applicable under field
conditions, although requiring relatively expensive portable equipment (Buguet, personal communication). Polysomnographic recordings appear to be potentially useful in monitoring the therapeutic response to melarsoprol (or other second stage drugs) and in identifying patients at risk of developing ES.

6.6 Risk factors for the encephalopathic syndrome

The interest for clinicians in the determination of risk factors for ES is related to the possibility of predicting which patients are at risk of developing ES. At the preventive and therapeutical level, clinicians need valid evidence regarding the efficacy of interventions designed to modify the risk of ES and to reduce case fatality rate once ES develops. Unfortunately, the acquisition of robust evidence regarding risk factors for ES was limited by the reduced number of studies specifically designed to look for them and by the deficient methodology applied in the majority of the studies. These limitations are also related to the frequently low statistical power and to the heterogeneity of the statistical methods used for evaluation of results. Thus, the evidence obtained in the systematic review most frequently derives from descriptive studies and is often circumstantial. Additionally, the inherent complexity of ES etiology and management leaves room for the existence of many confounders.

We grouped the obtained evidence for risk factors for ES (incidence risk) and for ES case fatality rate (CFR) in three categories:

1 - Validity of evidence substantial/convincing

- Gender, ethnic group and treatment schedule appear not to influence the incidence risk or the CFR.

- Concomitant infection appears to influence the incidence risk and the CFR.

- Administration of BAL appears to influence the CFR.
Observations:

Gender has no role in incidence risk or CFR. One exception is however observed in the specific setting of “epidemic” ES, where incidence risk and CFR appear to increase in males. Confounder: factors precipitating epidemic ES are unknown and could differently affect males (for instance: alcohol consumption).

Limited data is available for the role of the ethnic group, but not a single reference to this parameter in incidence risk or CFR was found.

Evidence from the IMPAMEL studies indicates that the incidence risk is independent of the melarsoprol treatment schedule.

Evidence from the IMPAMEL studies indicates that concomitant infection, mainly malaria, increase the incidence risk of ES and CFR. Circumstantial evidence indicates that concomitant Influenza virus infection precipitates ES.

Evidence from one correctly powered study shows a deleterious role of BAL on CFR.

2 - Validity of evidence probable/questionable
• Age, the form of HAT (Rhodesiense or Gambiense), the type of ES (coma, convulsions, mental) and alcohol consumption appear to influence the incidence risk or the CFR.

Observations:

Infants appear to have a lower incidence risk and a CFR similar to adults. Available data for parameter age is quantitatively limited.

Rhodesiense HAT appears to be associated with a higher incidence risk of ES and with a higher CFR. Possible confounders: definition of ES and attribution of the cause of death less clear in patients with Rhodesiense HAT
due to the more severe clinical expression of the disease. The observed extreme fluctuations in incidence risk and CFR observed in both forms of HAT may similarly reflect the same type of biases.

The coma, convulsions and mental types of ES appear to be respectively associated with a decreasing CFR. Possible confounders: overlapping of the three types of manifestations in the same patient appears to be the most common type of manifestation of ES and the clear separation between the 3 types of ES appears to constitute an observational bias. Mortality in coma and convulsion patients may be associated with problems in management of these conditions (coma may be iatrogenic) or the type of manifestation may be inherent to the different grades of severity of the phenomenon itself. The number of pathological studies is severely limited.

Evidence for an increased incidence risk associated with alcohol consumption during or after melarsoprol treatment is circumstantial but the general opinion is consensual.

Thiabendazol and praziquantel administration appears to increase the incidence risk. The statistical methodology of the only study describing this interaction is questionable (impossible to determine the p value).

3 - Validity of evidence conflicting/unreliable

- The precise influence of the following parameters on the incidence risk and/or CFR could not be determined: general status on admission, laboratory markers of HAT, and interventions for prevention and management of ES.

Observations:

General status is a subjective evaluation that combines the nutritional status, the intensity of symptoms and signs and occasionally of the biological
markers associated with disease severity. Conflicting results among the correctly designed and sized studies exist.

Laboratory markers of HAT severity include: the number of WBC in CSF, and the presence of trypanosomes in blood, lymph and CSF. Conflicting results between the two main correctly designed and sized studies exist.

Interventions for prevention of ES: no correctly designed trials exist except for the use of corticosteroids and these show conflicting results. A consensual opinion on the protective role of the ”patient preparation” exists. Potential confounders: high number of interventions, usually applied in combination; type of corticosteroid and dosage variable.

Interventions for management of ES: no correctly designed trials exist. The use of corticosteroids is consensual but no indication on the best drug and dosage exists. Data on the role of interventions for management of convulsions and coma is precarious.

Overall, the results of the systematic review in regard to the determination of risk factors for ES do not bring new evidence to the currently accepted views. What the review does show is the existence of many biases and the deficient methodology in the literature. Conflicting results in apparently adequately powered studies may reflect the multi-factorial nature of the phenomenon, the difficulties in applying homogeneous definitions or the multiplicity of interventions.

The number of the patients needed to reach statistical significance depends on the baseline incidence risk of ES. If we consider for instance the average ES incidence we found, to detect a significant decrease in incidence risk for one single intervention requires more than 200 patients per study arm. Pooling studies with different methodologies in the logistic regression we performed failed to show that interventions for the prevention or reduction of CFR are valid. This finding may result from the use of a defective statistic model (too
many confounders) or reflect reality. Simply considering that the measures currently accepted as capable of modifying the incidence of ES are invalid is a challenging hypothesis.

From the practical point of view, the risk factors that could be clearly identified and that can be manipulated are related to the existence of factors that can act as a second factor in triggering ES or add to the toxicity of melarsoprol, namely concomitant disease, and possibly systemic anthelmintics and alcohol. The knowledge that the occurrence of ES is dose-independent and occurs in average 10.2 days after the first melarsoprol administration can help design preventive and diagnostic strategies aimed at this specific time-point.

6.7 PREVENTION AND MANAGEMENT OF THE ENCEPHALOPATHIC SYNDROME

6.7.1 Prevention

Although patient preparation is not uncommon in Oncology, sleeping sickness is a unique example of a lethal infectious disease in which the administration of the indicated curative drug is postponed in order to prepare the patient for specific chemotherapy. Sleeping sickness is a disease of tradition. The rationale for this patient preparation was empirically established and is based on certain assumptions:

1 - HAT patients are immunosuppressed. Evidence for immunological imbalance in humans derives from the extrapolation of studies on the pathogenesis of African trypanosomiasis performed in animal models or in observations on animal diseases. However, studies demonstrating the existence and establishing the clinical characteristics of this hypothetical immune depression in humans are lacking. Malnutrition affects patients in advanced late stage HAT, adding to the hypothetically existing immunosuppression. Depending on the epidemiological situation and screening activities on a given HAT focus, the percentage of patients observed in this advanced stage of disease is limited. The Rhodesiense form
of HAT usually has a more severe impact in the patient’s condition on admission. However, the impact of HAT in nutritional status is badly studied, in particular in patients that are not obviously underweighted or anemic.

2 - HAT patients are often infected by additional infectious pathogens including parasites that have similar epidemiological distribution, like *Plasmodium* sp, *Filaria* sp, *Schistosoma* sp, and intestinal helminths and protozoan. However, the true incidence and impact of concomitant infections in HAT patients is badly studied. There are severe limitations on the availability of the diagnostic tests needed for identification of these pathogens in the field.

Failure to demonstrate that any of the prophylactic interventions in ES is effective in the logistic regression we performed is probably related to the multiple factors associated with the variability in patient presentation. The use of medications or other measures in HAT must not be seen as a similar procedure for all patients and should rather be tailored for specificities in the epidemiological context and in the clinical status of the patient.

The role of first stage trypanocidal drugs given before melarsoprol in reducing fever associated with the first administration of the arsenical seems obvious. This effect is described as more pronounced in Rhodesiense HAT, where parasitemia is frequently high. However, in an experimental study in 14 HAT (*T.b. rhodesiense*) patients receiving melarsoprol, fever after the first dose was correlated to cerebrospinal fluid (CSF) protein content and to CSF leukocyte cell count but not to parasitemia (Whittle and Pope, 1972). It is questionable whether pentamidine or suramin are able to promote CSF normalization in a sufficiently fast manner as to consequently reduce melarsoprol-induced fever in a significant way.

An example of the complex and multifactorial issues related to ES prophylaxis comes from the use of the antimalarial drug chloroquine. Chloroquine has well known systemic and CNS anti-inflammatory effects.
Chloroquine shows a dose-dependent ability to reduce pro-inflammatory cytokine production in human whole blood but also to induce expression of pro-inflammatory cytokines in astroglial cells in vitro (Karres, Kremer et al., 1998), (Park, Kwon et al., 2003). NO appears to mediate this cytokine imbalance (Park, Choi et al., 2004). The role of this drug in HAT patients is also questionable in areas of defectively know Plasmodium resistance to antimalarials. Furthermore, the issue is complicated by the difficulties in the interpretation of a positive thick smear for Plasmodium in hyper-endemic areas. This is illustrated by the finding of a positive Plasmodium thick smear in 14 out of 16 ES patients with fever in Angola (IMPAMEL I). Since all patients had received a standard full course of chloroquine before melarsoprol, the significance of this finding is not entirely clear (Blum, Nkunku et al., 2001). Nevertheless, chloroquine routine use in second stage HAT is accepted as good clinical practice and is recommended in many protocols.

It seems reasonable to think that improving the patient’s nutritional status may contribute to a successful outcome. Malnutrition causes depression of cell-mediated and humoral responses (Zumla, Sa'adu et al., 2002). Except in very advanced cases with severe CNS manifestations, normalization of food intake rapidly follows the establishment of successful anti-trypanosomal therapy. In the rat model weight loss appears to be an indicator of passage into neurological disease. Weight gain is an indicator of successful therapy in this model (Darsaud, Chevrier et al., 2004). Supplementation with retinyl palmitate ameliorates the resistance to trypanosomal infection in rats, increasing the antibody response to challenge and the lymphocyte count in blood (Ihedioha, Chineme et al., 2003). Thus it seems important to make sure that patients have access to a balanced diet with the correct amount of calories and macro and micronutrients, which is difficult to achieve in rural African health facilities. Supplementation of vitamins has a limited value in the absence of global nutritional improvement. Food supplementation would also have to consider the cultural characteristics of the population (i.e. milk may not be accepted). Nutritional studies that may help identify the most
common nutritional deficiencies associated with second stage HAT and its immunological impact are lacking.

The administration of anthelminths can help correct anemia through elimination or decrease in parasite-related iron and micronutrient expolation. The cost effectiveness and clinical benefits of the indiscriminate administration of mebendazol (the most widely used drug for this purpose) versus the identification of specific intestinal parasitosis followed by correct treatment remains to be determined.

6.7.2 Management

Besides of the intrinsic mortality of the phenomenon, one of the major determinants of CFR in ES must be the medical context where it occurs. In the African rural setting where HAT patients are managed, access to drugs is limited and intermittent. Life-supporting consumables and equipment for management of critically ill patients are usually missing or non-operational. Even when good quality medical and paramedical know-how is available, which is not frequently the case, staff is often overworked and unable to devote sufficient additional time to the demanding care of a convulsing or comatose patient in a critical condition (personal observations). This enhances the relevance of obtaining clear answers to the classical therapeutical question: how to select treatments to offer patients that do more good than harm?

BAL (dimercaprol) administration, which requires multiple intravenous applications, has been shown to significantly increase mortality in ES (Pepin, Milord et al., 1995). Melarsoprol is obtained by reacting melarsen oxide with BAL with the objective of reducing the toxicity of the arsenical. BAL is a rather toxic drug that has been shown to posses a low LD₅₀ in animals. Furthermore, BAL has also been shown to promote a redistribution of arsenic to the brain and testes, possibly enhancing melarsoprol passage through the blood brain barrier (Muckter, Liebl et al., 1997), (Jennings,
Atougia et al., 1996). Instead of reducing melarsoprol toxicity, the additional administration of BAL could in fact lead to a fatal outcome during ES by increasing the already existing levels of BAL to toxicity, by increasing the levels of melarsoprol in the CNS or through a combination of both mechanisms.

Adrenaline (in combination with BAL and other supportive measures) has been shown to be potentially useful in the management of ES in a small non-controlled trial. The basis for adrenaline administration in ES derives from the experience with this drug in the reactions observed during treatment of syphilis with arsenicals and penicillin (Sina, Triolo et al., 1982). In anaphylaxis and anaphylactoid reactions, adrenaline has been shown to antagonize the vasodilatating and pro-inflammatory action of the mediators generated (leukotrienes and prostaglandins) or released (histamine, tryptase, mast cell and basophil derived interleukine-4 and interleukine-13) during these phenomena (Austen, 2001). In mild reactions 0.01 mL/kg (0.3 to 0.5 mL for adults) of aqueous epinephrine (1:1'000, 1mg/mL) may be given subcutaneously and repeated as needed until resolution of anaphylaxis or signs of hyperadrenalism develop. Recently the intramuscular route of administration has been shown to be superior to the subcutaneous route (Simons, Gu et al., 2001). In life threatening reactions with severe hypotension or vasomotor collapse, 5 to 10 μg/min (0.3 to 0.5 mg, corresponding to 3 to 5 mL) of a more diluted epinephrine solution (1:10'000, 0.1 mg/mL) may be administered intravenously with close monitoring for side effects, including headache, tremors, nausea and cardiac arrhythmias (Ellis and Day, 2003). Any recommendation concerning adrenaline use in ES should to follow a correctly designed trial, including stratification for the severity of cardiovascular manifestations.

Promethazine is often cited in the management of ES, but no controlled trials are available. Promethazine is a phenothiazine drug that antagonizes histamine actions by interaction with H₁ receptors and is indicated in a variety of allergic
disorders, but has only a supplementary place in systemic anaphylaxis, in combination with adrenaline. Promethazine has potent antiemetic and sedative CNS actions and lowers the convulsive threshold. Simultaneous blockade of $H_1$ and $H_2$ receptors is currently recommended in anaphylaxis, based on the greater efficacy of this combination in animal models (Lieberman, 1990), (Ellis and Day, 2003). Future trials on ES should probably include both types of histamine antagonists.

Glucocorticosteroids have a potentially important place in the management of ES. Corticosteroids are useful in reducing and limiting the immunological phenomena involved in several autoimmune diseases and in reducing CNS damage associated with cerebral edema. Corticoids act by shutting down gene transcription for pro-inflammatory cytokine production. They help in temporarily repairing the blood brain barrier (Coyle, 2000). Steroid drugs have different anti-inflammatory and mineralo-corticoid (sodium retention) potencies. Optimal characteristics for a corticoid in ES should include a maximum anti-inflammatory and a minimum sodium retention action, to avoid aggravation of cerebral edema and fluid overload. It should be noted that the most commonly used drug in ES, hydrocortisone, has the highest sodium retention action. The ideal drug for ES treatment should be parenterally administered in order to efficiently and quickly obtain anti-inflammatory action. The duration of administration should be the shortest possible to avoid potentially severe side effects, such as gastrointestinal bleeding and additional immunosuppression. Corticosteroids antagonize the hypotensive effect of every class of antihypertensive drugs (including diuretics). When used in combination with diuretics there is an increased risk of hypokalemia. Use of steroids with acetylsalicylic acid and non-steroidal anti-inflammatory drugs carries an increased risk of gastrointestinal bleeding and ulceration (WHO, 2002). Salicylates for fever (or headache) control in ES may also be associated of an increased risk of hemorrhagic manifestations in the affected brain capillaries.
The choice of the optimal steroid drug, dosage and duration of administration in ES remains to be determined. Indications should come from the accepted use of steroids in cerebral edema, in viral encephalitis, in ADEM and in AHLE. For cerebral edema the currently used scheme consists in dexamethasone (0.5 to 0.6 mg/kg/day) divided in 4 or 6 doses (WHO, 2002). No randomized controlled clinical trials exist for the use of steroids in ADEM, AHLE or viral encephalitis. There is limited evidence that in these conditions high (5 to 10 mg/kg/day) and very high (1000mg/day for an adult) doses of methylprednisolone may bring better results (Schwarz, Mohr et al., 2001), (Nakano, Yamasaki et al., 2003). Correctly designed clinical trials are needed to establish the optimal use of steroid schemes in ES.

Concerning the value of drugs and measures needed to manage a convulsing or comatose ES patient the literature is surprisingly poor. General recommendations for the correct use of drugs to control convulsions in ES have been issued by the WHO (WHO, 1986).

For isolated convulsions and status epilepticus, phenobarbital and diazepam are exclusively cited, but we found extremely limited data concerning dosage. It is probably assumed that the use of these drugs in ES follows the accepted recommendations for this type of conditions. It should be noted that both drugs have a narrow therapeutic window and potentially severe iatrogenic effects, including respiratory depression and arrest. Incorrect, incautious or deficiently supervised use of phenobarbital and diazepam (especially in sequential combination) may be the source of additional problems for an already critical ES patient, including the induction of iatrogenic coma. Furthermore, interactions exist between phenobarbital, diazepam and other drugs potentially useful in ES. Chlorpromazine and haloperidol have antagonic actions in relation to phenobarbital, lowering the convulsive threshold. Phenobarbital accelerates the metabolism of corticosteroids, resulting in reduced effect. Diazepam interacts with promethazine, chlorpromazine and haloperidol, resulting in an enhanced sedative effect.
Diazepam enhances the hypotensive action of diuretics and almost any other antihypertensive drug. Cimetidine inhibits diazepam metabolism resulting in increased diazepam plasma concentration (WHO, 2002). The use of phenytoin or other potentially less toxic anticonvulsants has not been reported in HAT.

The data from Haller’s neuropathological study in ES suggests that hypoxic brain damage is probably a frequent feature in ES, especially when related to status epilepticus. Administration of oxygen is certainly useful in this condition but very rarely cited in the literature on ES. This may simply be related to the non-availability of medicinal oxygen in the majority of the health facilities treating HAT patients. Another important feature of Haller’s series is the existence of damage to the Purkinje cerebellar cells, suggesting that persistent hypoglycemia during ES may be a crucial factor. Hypoglycemia may be prevented by the routine initial administration of hypertonic (50%) glucose in water in bolus. Careful monitoring of fluid and electrolyte balance and caloric intake is critical to maintain correct hydration and kidney function, as well as in giving energetic support to the patient catabolic status.

The care of a patient in coma requires measures aimed at rapidly and assiduously assessing and controlling hypotension, hypoglycemia, hypoxia, hypercapnia, hyperthermia and electrolyte imbalance. The absence of minimally well equipped laboratory facilities in the context where HAT patients are managed may complicate the assessment of some of these parameters. However, careful observation of the patient cardiovascular and respiratory status and monitoring fluid input/output, although time consuming, may help prevent, identify, and treat cardiovascular and metabolic complications.

Controlling raised intracranial pressure must play an important role in the management of ES patients suffering from this condition. The dramatic impact of reducing raised ICP in ES is demonstrated by the anecdotic report
of a cisternal tap resulting in quick recovery of a normal level of consciousness in one patient (Onyango and Ogada, 1968). The administration of mannitol (an osmotic diuretic drug) in sufficiently large doses results in reduction of increased ICP within 15 minutes of the start of the infusion and the effect lasts for 3-8 hours after discontinuation. Achieving and maintaining a reduction in raised ICP with mannitol requires careful and frequent monitoring. Furthermore, mannitol produces circulatory overload due to expansion of extracellular fluid; in patients with a diminished cardiac reserve this may result in pulmonary edema (WHO, 2002). Thus, this drug should only be used in facilities where the capacity for diagnosing increased ICP and for monitoring mannitol effects exists. Hypertonic saline solutions have recently emerged as a potentially safer and more efficacious alternative to mannitol (Qureshi and Suarez, 2000), (Georgiadis and Suarez, 2003). Future clinical trials on the use of mannitol in the management of ES should include corticosteroids as the first option for reduction of brain edema and raised ICP, with mannitol or hypertonic saline solutions as a rescue measure in case of failure.

Although seldom cited in the literature, antibiotics should be useful in ES patients, especially in those surviving long enough to develop complications associated with coma or status epilepticus. The choice of antibiotics in the African health care setting is frequently limited, and more so in the rural context of HAT. In this context penicillin or another β-lactam are the most commonly available antibiotics. Antibiotic usage in ES needs correct evaluation. The choice of antibiotics to be included in future trials should take into account the severe limitations in availability of this type of drug in rural Africa.
7 CONCLUSIONS

The present systematic review on the literature on ES demonstrates that existing clinical studies on the subject are of heterogeneous and frequently of deficient quality, precluding the acquisition of consistent evidence on several critical aspects of ES. One of the main benefits of the review was to allow the many biases present in the literature to become apparent.

The systematic review helped obtain a more uniform definition of the encephalopathic syndromes.

The discussion of the etiology of ES helped to establish an analogy between ES and AHLE, leading to the hypothesis that a common pathogenesis mechanism may be present.

A comprehensive description of the potential risk factors was obtained. Concomitant infection appears as one the few risk factors that can be effectively modified.

The review shows that there is room for improvement in the prevention and management of ES. Correctly designed clinical studies are needed to test the several possible ways of optimizing interventions for the prevention and treatment of ES.

The present epidemiological situation offers the possibility of implementing correctly designed clinical studies to clarify many pending issues in sleeping sickness. As melarsoprol will probably remain in use for many years, studies aimed at increasing knowledge on the etiology, the definition, the risk factors and the prevention and management of ES are necessary and should be prioritized.
PART III
CLINICAL STUDY ON THE ENCEPHALOPATHIC SYNDROME

8 INTRODUCTION

8.1 RATIONALE

Despite the significant incidence and mortality associated with the encephalopathic syndrome during treatment of HAT with melarsoprol, which has a high impact in individual patients and seriously jeopardizes the efficacy of melarsoprol, sound scientific knowledge and solid and robust evidence regarding many critical aspects of ES remains to be acquired, as shown in the systematic review on ES.

In the absence of a sufficient number of anatomopathological studies, and of biological markers, characterization of ES is mainly clinical. Convulsions, coma, psychotic manifestations separately or in combination are said to characterize ES in its various forms. It is not clear whether these forms correspond to the same etiology or if they are different phenomena. The prognosis of the three hypothetical forms also varies according to the clinical manifestations, although it is not clear if this is inherently associated with ES itself or the iatrogenic result of interventions designed to prevent and treat ES. A clear definition of ES is needed to be able to establish the correct (differential) diagnosis and to guide the therapeutic approach of the condition.

Clinicians also need better knowledge on the risk factors for ES, in order to design preventive strategies or to selectively avoid melarsoprol administration in high risk patients whenever efornithine is available as an alternative drug. Evidence for risk factors is based mainly on retrospective studies and is bias prone. The few correctly designed studies show conflicting results. This may be associated with the insufficient statistical power of those studies but also with the heterogeneity of the statistical methods applied in the analysis of results. The inherent complexity of the phenomenon, resulting in many
variables being involved in ES, could additionally contribute to the observed difficulties in establishing the causality of relationships between the multiple factors involved. Correctly designed prospective studies are needed to help correctly determine the existence and weight of risk factors for the occurrence of ES and for ES case fatality rate.

Existing evidence indicates that immunological phenomena are involved in the triggering and pathogenic mechanisms of ES. It remains however to be determined why, how and under what conditions/circumstances the three main actors on the scene, i.e. melarsoprol, the parasite and the host interact to trigger ES only in selected patients. Knowledge on the etiology and pathogenesis of ES is crucial to establish the basis for scientifically based interventions for the prevention and therapy of ES.

In humans, the individual characteristics of the immune response are mainly related to the HLA complex. The encephalopathic syndrome, with its characteristics of an immune phenomenon occurring within the CNS, could be associated to the patient HLA type.

8.1.1 The HLA complex and disease

In humans, gene products of the Major Histocompatibility Complex (MHC) play a central role in the distinction between peptides derived from their own proteins (self) and those derived from foreign (nonself) proteins. The main function of MHC molecules is peptide binding and presentation of them to T lymphocytes. Human leukocyte antigens (HLA) are the designation for antigens that are products of the MHC. In our species, the HLA complex corresponds to the entire histocompatibility complex. MHC is located on the middle of the short arm of chromosome 6, where genes coding for HLA occupy a segment of approximately 4 million base pairs of DNA. The HLA complex contains over 200 genes, more than 40 of them encoding leukocyte antigens. Each person has two alleles for each locus or alternatively a pair of identical alleles. The genes are allelic and all alleles are co-dominant (Klein and
The massive sequencing project of a human MHC haplotype has been completed and the map positions of all of these genes are known (Anonymous, 1999).

The HLA complex is divided into three regions: class I, II, and III regions, as first proposed by Jan Klein in 1977. There has also been a suggestion for a class IV region located at the telomeric end of the class III region (Gruen and Weissman, 1997). The classical HLA genes associated with the immune response belong to Class I and to Class II (Figure 10).


Class I antigens are found on virtually any nucleated cell and on platelets. These molecules are heterodimeric polypeptides consisting of a heavy chain
bound to a $\beta_2$-microglobulin molecule. Genes at the HLA-A, B or C loci encode the heavy chain. Class II antigens are mainly expressed on antigen–presenting immune cells such as B-lymphocytes, T-lymphocytes, macrophages/monocytes, dendritic cells, and some endothelial cells. In the presence of INF-γ other types of cells can also express Class II molecules. Class II antigens are made of two ($\alpha$ and $\beta$) polypeptide chains that are encoded by genes at the HLA-D locus. This region is divided into subregions denominated HLA-DR, DQ and DP (Klein and Sato, 2000) (Figure 11).

Class I and II molecules bind peptides derived from proteins of intracellular and environmental origin, respectively. This binding between endogenous and exogenous molecules provides a context for the adequate recognition of nonself antigens by T-lymphocytes. CD8+ cytotoxic T-lymphocytes

recognize the products of intracellular degradation of viral proteins plus class I molecules displayed on cell surface while CD4+ helper T-lymphocytes recognize fragments of proteins (bacterial or parasitic) plus class II molecules. The process of capture and biosynthetic maturation of exogenous peptides provides a continuously updated display of Class I and Class II molecules for scrutiny by T-cells. Endocytosis, phagocytosis, macropinocytosis of the peptides and their exportation to the surface of the cell is a continuous and complex multi-step process (Watts, 1997, Klein, 2000).

The class III region has the highest gene density but some of the genes are not involved in the immune system (Gruen and Weissman, 1997). Among the genes which are of interest, HSP70, TNF, C4A, C4B, C2, BF and CYP21 should be mentioned. An important feature of HSP70 (Heat Shock Proteins) alleles makes this locus a useful one in disease association studies. They show strong linkage disequilibrium (LD, see below) with HLA-DR alleles (Partanen, Milner et al., 1993). TNF-α and TNF-β genes encode cachectin and lymphotxin-a molecules, respectively. C2, C4A and C4B are the genes for some of the complement proteins, whereas BF codes for factor B which is also involved in the immune response. The CYP21 isoform of Cytochrome P450 is the gene for 21-hydroxylase which is an important enzyme in corticosteroid metabolism.

One of the important characteristics of the MHC is its extreme polymorphism (genetic variants that are found in a significant proportion of the general population). Among the expressed loci, the MHC has the greatest degree of polymorphism in the human genome. MHC polymorphism is so intense that it is theoretically possible for each human to possess a different set of MHC alleles. This great diversity of the HLA region is thought to arise from host challenge with infectious agents as a survival strategy (Segal and Hill, 2003). Another highly relevant feature of the MHC antigens is their co-dominant expression and the fact that the MHC is inherited en bloc as an haplotype, with the exception of the rare recombinational events. Despite the
enormous number of alleles at each expressed loci, the number of haplotypes observed in populations is much smaller than theoretical expectations: certain alleles tend to occur together on the same haplotype rather than being randomly segregated together. This is called linkage disequilibrium (LD) and can be quantitated by a differential $\Delta$ value (Begovich, McClure et al., 1992).

The initial interest in HLA was the improvement of donor/receptor compatibility in clinical transplantation, but the biological and clinical importance of HLA soon became apparent. The fields of HLA and disease susceptibility (association studies) and HLA and evolution and anthropology developed (Marsh, Parham et al., 2000). Indeed, most of the studies on HLA were carried out on large urban populations of industrialized countries. The knowledge of HLA types in Central African populations is comparatively very limited. However, nucleotide diversity within African populations tends to be greater, possibly indicating the recent African origin of all modern humans (Kwiatkowski, 2002).

8.1.1.1 Associations between the HLA complex and disease

Two types of statistical methods have been used for the direct identification of human genetic determinants of disease: linkage analysis and association studies.

Linkage analysis has proved to be very useful in identifying a region on the genome that is transmitted within families along with the disease phenotype under scrutiny. Several regions in the genome have been associated by linkage analysis with simple Mendelian inherited diseases (monogenic diseases), such as cystic fibrosis. This approach is however much less efficient when applied to complex diseases, where inheritance is influenced by multiple genetic and environmental factors.

Association studies have been classically performed to identify genetic polymorphisms that are associated with complex diseases. The usual approach is to compare, in a case control setting, unrelated individuals that
are either affected (cases) or unaffected (controls) by the disease phenotype. Significant differences in allele (or genotype) frequencies in cases and controls are taken as evidence for involvement of the allele or genotype in disease susceptibility. Since in the MHC both alleles are co-dominant and contribute to the phenotype equally, it is important to investigate the genotypes in disease association studies rather than the alleles on their own. If susceptibility to a disease is a recessive trait, allelic association studies may not yield a positive result. This has important implications in disease association studies: a haplotypical association is usually stronger and more meaningful than an allelic association. The ultimate goal of all association studies is to gain insight into the molecular and cellular basis of disease pathogenesis, in order to improve preventive strategies, diagnostic tools and therapies (Silverman and Palmer, 2000).

Many more diseases have been associated with the HLA complex than with any other part of the human genome. Diseases that are associated with HLA include specially those that are caused by a chronic state of inflammation/autoimmunity. The list of these pathologies includes insulin-dependent diabetes mellitus, ankylosing spondylitis, systemic lupus erythematosus, pemphigus vulgaris, rheumatoid arthritis, myasthenia gravis, multiple sclerosis, Goodpasture’s syndrome, and Graves’ disease. Since only a minority of people with the genetic predisposition develops overt disease, it is believed that the immune response to environmental factors, such as infections, in combination with the genetic background, play a major role in autoimmune diseases associated with HLA (reviewed by Marsh, Parham et al., 2000).

A link between HLA and infectious diseases has been established for viral, bacterial, fungal and parasitic infections. The list of statistically significant associations is presently quite extensive. Viral diseases include those associated with Dengue fever virus, Human immunodeficiency virus type 1, Hepatitis B and C virus, Epstein-Barr virus, and Puumala virus. Bacterial
diseases include tuberculosis, leprosy, Reiter disease and acute anterior uveitis. Fungal diseases include paracoccidioidomycosis and probably cryptococcosis. Statistically significant associations between HLA and susceptibility and/or resistance against malaria, scabies, and schistosomiasis are established (reviewed by Singh, Agrawal et al., 1997) and Kwiatkowski, 2002).

As for the infections associated with pathogens of the Trypanosomatidae family, a link has also been established for Leishmania-related diseases such as diffuse cutaneous leishmaniasis (HLA A11, B5, B7), localized cutaneous leishmaniasis (HLA Bw22, DQw3) and visceral leishmaniasis (HLA A6). The chronic stage of Chagas disease, associated with *T. cruzi* infection, is characterized by a progressive cardiomyopathy with severe arrhythmia and/or congestive heart failure. The tendency to develop this severe form of the disease has been linked with the increment of the DRB1*01 DQB1*0501 haplotype and with the DPB1*0401 allele (Colorado, Acquatella et al., 2000). In a previous study, susceptibility to Chagas disease was linked to the A30 antigen, while DQB1*06 conferred protection, regardless of the clinical presentation of the disease (Deghaide, Dantas et al., 1998). More recently the DRB1*14- DQB1*0301 haplotype was shown to be protective regarding *T. cruzi* infection in a highly endemic area. No differences in allelic or haplotypic distribution between asymptomatic and cardiomyopathic patients were observed in this population (Nieto, Beraun et al., 2000).

In human African trypanosomiasis, the genetic predisposition to infection has been discussed based on the phenomenon known as “house effect” and “family contamination” in human population (Gouteux, Noireau et al., 1988) and on the resistance/tolerance towards African trypanosomes of cattle (Murray, Trail et al., 1984), (Hanotte, Ronin et al., 2003) and mice (Greenblatt, Diggs et al., 1984), (Pinder, 1984), (Kemp, Iraqi et al., 1997). No study with human genetic material has however been performed to investigate a relationship between HAT and HLA. It is generally accepted that humans
infected with pathogenic subspecies of *T.b. brucei* are equally susceptible to
develop disease.

### 8.1.2 Methods for HLA typing

The inadequacy of the classic serological and cytotoxicity methods for HLA
typing became more and more apparent during the 1980s, when biochemical
and molecular biology techniques progressively revealed the high
heterogeneity of HLA polymorphism, especially for Class II antigens. More
precise DNA-based typing methods, namely polymerase chain reaction with
sequence-specific oligonucleotide (PCR-SSP) primers can directly assess
DNA sequences instead of the proteins they encode (Marsh, Parham et al.,
2000). The basic sequence of events for this type of method is shown in
figure 12.
A comparative study between classical serological HLA-A and HLA-B class I typing and PCR-SSP typing in 421 Black individuals showed a 36.3% discrepancy in all individuals tested, much higher than the 8.5% value observed in Caucasians (Mytilineos, Lempert et al., 1998). The degree of discrepancies between the two methods found in a recent study in a mixed Brazilian population (whites, blacks, mulattoes and orientals) consisting in 1160 renal transplant patients typed for Class II HLA-DR was not so intense, but serologic methods still failed to assign HLA-DR specificities more often than oligotyping (Ferraz, Saber et al., 2002). Both studies clearly indicate that
molecular techniques are more accurate than serological typing and can improve HLA matching in clinical transplantation.

8.2 Objectives
The purpose of this component of this thesis was to design and conduct a case-control clinical study with prospective clinical data collection on the encephalopathic syndrome during treatment of late stage Gambiense HAT with melarsoprol.

The main goals were to provide clinicians with a more precise and consistent definition of the syndrome, to identify and quantify clinical risk factors for ES and for ES case fatality rate and to determine whether an association between ES and Class I and II HLA antigens exists.

Additional aims were to improve the diagnosis and management of ES and, should an association between ES and HLA be found, to elaborate the basis for the identification of a genetic marker for ES susceptibility and to gain insight into the etiology and pathogenesis of ES.

9 Materials and methods
9.1 Study design
We designed a case-control, non-blinded, non-randomized, non-interventive, multi-centre study, with prospective clinical data collection, comparing patients in late stage Gambiense HAT treated with melarsoprol and developing or not developing an encephalopathic syndrome.

The study protocol was designed according to established Good Clinical Practice international recommendations. Study protocol development was carried out in close collaboration with the Swiss Tropical Institute statisticians at the Biometrics Unit and with the coordinators of the National Sleeping sickness programs in Angola (Instituto de Combate e Controlo das Tripanossomíases, ICCT) and in the Democratic Republic of Congo (Bureau Central de la Trypanosomiase, BCT, presently denominated Programme
National de Lutte contre la Trypanosomiase Humaine Africaine, PNLTHA). The study protocol was embedded in the framework established by the IMPAMEL II program.

Participants were enrolled in HAT treatment centres in both countries. Participating centres were chosen in agreement with the national coordinators of the ICCT and PNLTHA. The choice was determined by the existence in the participating centres of a full-time clinician, in order to allow access to adequate clinical documentation. Chosen centres treated a significant number of late stage HAT patients. The choice was also determined by the existence of reasonably good accessibility and communication possibilities with the treatment centre. The number of centres was estimated in order to allow the enrolment of the defined number of cases within a year after initiation of the study. The estimation also took into account the possibility of discontinuation of participation of some centres due to social instability or military activities, which would prevent the continuation of the study.

Since data on genetic predisposition to ES is non-existent, no model was available to explore the relationship between disease phenotype and patient genotype. The design of the proposed study was nonparametric, consisting in testing whether or not the alleles of a given marker are distributed at random in patients suffering from ES (Abel and Dessein, 1998).

Sample size for the study was estimated at a total number of 100 cases and 300 controls. This number represented a compromise between the necessary statistical power, the known incidence of ES and the expected limitations in study protocol performance in the field.
9.2 Study Population

9.2.1 Inclusion criteria

Inclusion criteria for cases and controls were defined as follows:

- The patient has late stage *T. b. gambiense* disease diagnosed by:
  - trypanosomal infection diagnosed either serologically or parasitologically plus
  - trypanosomes in CSF and/or CSF cell count > 5 cells and/or
  - clearly defined neurological signs and/or
  - increased levels of IgM in CSF
- The patient is hospitalized and treatment with melarsoprol is ongoing
- The patient is conscious (Coma Score ≥ 9 in the modified Glasgow Coma Scale) before the start of melarsoprol treatment
- The patient is not convulsing before the start of melarsoprol treatment
- Mental changes of the psychotic type are not reported in the anamnesis or observed before the start of melarsoprol treatment
- Appropriate informed consent is obtained

A patient was considered as an encephalopathic syndrome case when the following inclusion criteria were fulfilled:

- Onset of coma and/or
- Onset of convulsions and/or
- Onset of severe mental symptoms, such as psychotic reactions and/or abnormal behaviour (aggressivity, severe confusion or disorientation) requiring a therapeutic intervention

9.2.2 Exclusion criteria

Exclusion criteria for both cases and controls were defined as follows:

- The patient receives combination therapy, i.e. melarsoprol plus any other anti-trypanosomal drug
- Informed consent is not obtained
- Patient follow-up is not assured for 30 days after discharge

9.3 Study Methodology and Protocol Implementation

In order to allow the prospective acquisition of clinical data from the participants, a questionnaire (Case Report Form, CRF) was created in collaboration with the Biometrics Unit of the Swiss Tropical Institute (STI),

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Basel. To obtain a maximum of homogeneous definitions and to facilitate acquisition and subsequent data extraction, parameters in the CRF were presented mainly in a structured form. Additional unstructured detailed and potentially valuable clinical data concerning the characteristics of ES was also requested. The CRF was translated to Portuguese and French and discussed and adapted to the needs of the National coordinators of the ICCT and PNLTHA (Appendix 1, French version of the Case Report Form). All clinical data was obtained by the local investigator with the help of the principal nurse, directly from the patient and/or from relatives and from the treatment centre laboratory.

For every patient diagnosed with late stage sleeping sickness and treated with melarsoprol in the participating centres, the CRF was used to prospectively collect and record epidemiological, clinical and laboratory data as well as the characteristics of the treatment, including interventions aimed at preventing ES. Should the patient develop an ES, the characteristics of the clinical presentation, management and final outcome of the syndrome were additionally inserted into the CRF. Patients not developing ES were enrolled as controls. For each case, three controls were enrolled. The controls were selected as the next three HAT patients following an ES case who underwent final discharge examination after having successfully completed treatment with melarsoprol in the same centre.

In every participating centre the management of HAT patients (i.e. admission, diagnosis, treatment, discharge, and follow-up) followed the Guidelines established by the ICCT or the PNLTHA. When a case of ES was diagnosed, melarsoprol treatment was ceased and the complication was managed according to the existing Guidelines of the ICCT or the PNLTHA for this condition.
An emergency diagnostic blood test for *Plasmodium* was mandatory in patients developing ES. The result of this test was made available to the clinical staff as soon as possible for adequate management of the patient.

Capillary blood was collected onto special paper filter cards (Generation® Sample Collection Cards, Gentra Systems, MN, U.S.A., see HLA typization section below) for HLA typing, according to given indications. HLA sample collection was performed when the diagnosis of the condition was established in ES cases and at discharge for controls. HLA blood samples in filter paper were attached to each patient CRF, stored according to instructions and periodically shipped to the principal investigator via the ICCT or the PNLTHA.

Apart from the introduction of the Case Report Form and the collection of the blood sample for *Plasmodium* testing and HLA, no additional changes in any other routine procedures were introduced, and no additional staff was required for the study.

9.3.1 *Study implementation*

The study protocol was initiated in June 2002 in Angola and in August 2002 in the DRC. Study protocol implementation was optimized with the help of two workshops performed in Kinshasa (August 2002) and Luanda (September 2002). Theoretical and practical aspects related to HAT treatment and ES in general and with the study protocol in particular were extensively discussed with the clinicians from all participating centres. This allowed a better standardization of the clinical definitions used in the CRF.
9.3.2 Study sites

The study protocol was implemented in the following HAT treatment centres (Figure 13):

- Angola:
  - Centro de Referência e Investigaçāo de Viana (CRIV), Viana (Luanda)
  - N’Dalatando (Kuanza Norte Province)
  - Uige (Uige Province)

- DRC:
  - M’Buji Mayi (Kasai Oriental Province)
  - Maluku, Kinshasa
  - Centre Neuro-Psycho-Pathologique (CNPP), Kinshasa

![Figure 13 - Study sites](image)

9.3.3 Study follow-up

Two monitoring visits were performed, one to Angola in March 2003 and one to the DRC in April 2003. Study follow-up was otherwise mainly...
obtained by periodic telephonic and email contact with the ICCT and PNLTHA, so that the staff executing the study could receive appropriate instructions and back-up. Additional on-site monitoring was kindly performed by Dr. Christian Burri, Dr. Gabriele Pohlig (STI) in the DRC and by Prof. Lars Rombo (Infektionskliniken, Malarsjukhuset, Eskilstuna, Sweden) in Angola, while on mission to these countries. Patient enrolment was terminated in all participating centres in November 30th 2003.

9.3.4 Ethical aspects

Study initiation took place only after official ethical clearances were obtained from the Ethical committees of both cantons of Basel (Switzerland), Luanda (Angola) and Kinshasa (DRC).

All participants were asked to sign the Informed Consent Form (Appendix 2, Portuguese version). Patients suffering from an ES event were frequently unable to give informed consent because of a decreased level of consciousness and/or mental changes. Therefore the accompanying family of the ES patient was also involved in the consenting process. Control patients were directly asked to sign the informed consent form at the end of melarsoprol treatment. If their status did not allow giving consent, their family was also involved.

The implemented study protocol is a “research combined with medical care” non-therapeutic study. Expected benefits for the patients included a better over-all clinical management associated with a more complete and structured medical anamnesis and the identification and treatment of a potentially severe malaria episode. The risks and burdens for the patients were those associated with a capillary blood sample collection and are considered a “minimal differential risk”. No changes in patient management were introduced, except those resulting from a malaria diagnosis in case of an ES event.

Following the results of HLA typization, additional investigations potentially useful for the clarification of the etiology of ES were considered ethically
valid and may in the future be conducted in the collected samples. Further undisclosed investigations were considered ethically unacceptable.

9.4 DATA MANAGEMENT
Data on Case Report Forms was checked for inclusion and exclusion criteria and for completeness and consistency. Missing data and clarifications were obtained from the local investigators via the ICCT or the PNLTHA whenever needed.

Data from the CRFs was subsequently double-entered into two databases prepared in EpiData (http://epidata.dk/), one for the part of the questionnaire common to cases and controls and the other for the characterization of the encephalopathic syndrome in ES cases.

The databases were cleaned from inconsistencies, merged and exported to the statistical software program “Statistical Package for the Social Sciences” (SPSS for Windows, Rel. 15.5.0. 2002. Chicago: SPSS Inc.) for analysis.

9.5 HLA TYPIZATION
9.5.1 General aspects
9.5.1.1 Typing Laboratory
HLA typing was performed at the Molecular Genetics Laboratory of the Centro de Histocompatibilidade do Norte (North Histocompatibility Centre, CHN), Porto, Portugal. This Centre is certified by the European Federation for Immunogenetics for histocompatibility testing since October 2002. Certified services include organ transplantation (renal and non-renal), haematopoietic progenitor cell transplantation and disease association studies. Certified techniques include serology (CDC, ELISA and flow cytometry), DNA typing (2 and 4 digits) and mixed lymphocyte culture (MLC).

9.5.1.2 Sample collection and handling
Blood samples for HLA typing were collected by digital puncture. Capillary blood was directly spotted onto special filter paper cards (Generation® Sample
Collection Cards, Gentra Systems, MN, U.S.A.). Blood spotted cards were carefully dried before inserting them into sealable plastic bags, and stored at room temperature. Sample storage time before DNA purification ranged from 4 to 12 months.

After each patient sample was successfully typed for HLA Class I and II antigens, the cards with the remaining blood were kept at -20°C for further needs.

9.5.1.3 DNA purification method

DNA purification was performed according to the protocol indicated in the Generation® Capture Card Kit for purification of DNA (Gentra Systems, MN, U.S.A.) from Generation® Sample Collection Cards. The Generation® system is based on reagents that lyse cells and capture and release the purified DNA, while proteins and other contaminants are selectively removed.

A hole punch was used to obtain 3 mm blood spotted disks from the cards. The disks were placed in 1.5 ml Eppendorf® tubes for manual processing. Each tube received 150 µl of the supplied “DNA Purification Solution” and was incubated for 15 minutes at room temperature. The solution was mixed by pipetting up and down 3 times. Finally, as much solution as possible was removed from the tube. Three washes using the “DNA Purification Solution” were performed for each disk. After adding 150 µl of the supplied “DNA Elution solution” the disks were incubated for 15 minutes at room temperature. The solution was pipetted up and down and as much solution as possible was removed. At this point, DNA remains bound to the disk. One hundred microliters of the “DNA Elution solution” were again added to each tube containing the purified disk; these were incubated at 99°C in a dry block heater for 15 minutes and cooled down to room temperature. This step allows DNA to be released. The content of the tube was pipetted up and down 15 times and the eluted DNA was transferred to clean tubes for DNA amplification.
DNA concentration in the eluate was determined by UV spectrophotometry in a Genequant™ II RNA/DNA Calculator (Amersham Biosciences, U.S.A.). Absorbance was measured at 260 nm. DNA concentration was generally in the 40-50 ng/μl range.

9.5.1.4 Typing methods

Samples were typed for Class I HLA-A, HLA-B and HLA-Cw molecules and for Class II molecules of the HLA-DRB1 category using two different commercially available PCR-based reverse line blot assays that detect specific target DNA sequences by means of multiple immobilized sequence-specific oligonucleotide (SSO) probes (Saiki, Walsh et al., 1989), (Buyse, Decorte et al., 1993), (Bugawan, Apple et al., 1994), (Begovich and Erlich, 1995). The tests are based in PCR target amplification, followed by hybridisation of the amplified products to an array of immobilized sequence-specific oligonucleotide probes and detection of the probe-bound amplified product by colour formation. The principle and sequence of events of the methods used (similar for Class I and class II HLA types) are schematically shown in figure 14. The example shows a method for Class I and Class II simultaneous typing. In the present study two different tests based on similar methods were used for Class I and Class II typing. Detailed description of the procedures are found under “Typing for Class I molecules”. Figure 15 shows in detail the amplified target DNA detection by chromogen colour change.
Figure 14: Schematic representation of a PCR-based reverse line blot assay using multiple immobilized sequence-specific oligonucleotide probes for identification of Class I and Class II alleles. Source: www.innogenetics.com.

Figure 15: Detail of the method for detection of amplified DNA target by colour formation. Source: www.innogenetics.com.
9.5.2 Typing for Class I molecules

9.5.2.1 Typing method

For HLA-A, HLA-B and HLA-C typing the Dynal RELITM SSO HLA Test (developed by Roche Molecular Systems, Inc. and manufactured by Dynal Biotech Ltd, Wirral, U.K.) was used. This PCR-based reverse line blot assay provide low to intermediate resolution typing of the HLA-A, HLA-B and HLA-Cw genes.

The specific target DNA sequences are the polymorphic second and third exons of the respective genes. The “master mix” contains a primer pair for exon 2 and a separate primer pair for exon 3. These two exons are co-amplified. For HLA-A, the target sequences are 480bp (exon 2) and 314bp (exon 3). For HLA-B the target sequences are 376bp (exon 2) and 335bp (exon 3). For HLA-Cw the target sequences are 410bp (exon 2) and 519bp (exon 3). The tests have the potential to identify all known HLA-A, HLA-B and HLA-Cw alleles.

All pre-PCR and post-PCR amplification procedures were performed using micropipettes with plugged (aerosol barrier) tips.

9.5.2.2 PCR Amplification reaction

The purified DNA solution (3.8 µl) was transferred to previously labeled 0.2 ml PCR tubes (AB gene®, Advanced Technologies Ltd, Epsom, Surrey, U.K.). Seven point five microliters of the “Master Mix” (a Tris-HCl solution containing 10% glycerol, 100nM KCl, <0.001% dATP, dCTP, dGTP, dUTP, biotinylated primers, <0.01% AmpliTaq®, 0.05% sodium azide), and 3.8 µl of the “6.0 mM MgCl₂ solution” (a 6.0 mM magnesium chloride solution containing 1% Proclin®300 as a preservative) were added. Based on previous experience with this type of commercial assay and to increase performance, 2.5 units of Taq polymerase (Platinum® Taq DNA polymerase, Invitrogen Corp, U.S.A.) were added to each tube before centrifugation.
Samples were then placed in a Thermalcycler (Perkin-Elmer 9600-1 or 9600-2 GeneAmp PCR System, Perkin Elmer Cetus, Norwalk, CT, U.S.A.) programmed for 35 cycles as recommended by the test kit manufacturer (15 seconds at 95°C, 45 seconds at 60°C, 15 seconds at 72°C and again 5 minutes at 72°C). Denaturation at 95°C separates the double stranded DNA and exposes the target sequences to the primers. At 60°C, the biotinylated primers anneal to their targets. At 72°C and in the presence of excess deoxynucleoside triphosphates (dNTPs), the thermostable recombinant *Thermus aquaticus* DNA polymerase extends and elongates the annealed primers along the target templates to produce a biotinylated DNA sequence (an amplicon).

Control of the PCR reaction was performed by adding a molecular weight marker to a solution containing 5 μl of each sample and 2 μl of “loading buffer” that was placed in wells of a 2% (w/v) agarose gel containing 0.001 % ethidium bromide and submitted to electrophoresis at 150 volts for 15-20 minutes. After fragment migration the existence of the correct molecular weights for each locus was checked against the molecular weight marker using a UVitec STX-40.M Transilluminator (Cambridge, U.K.) connected to a Kodak Digital Science™ Electrophoresis Documentation and Analysis System 120 (Eastman Kodak Company, U.S.A.).

9.5.2.3 Hybridisation reaction

Hybridization was performed using an AutoLIPA (Innogenetics, Ghent, Belgium) automated assay processor, programmed to fit the Dynal RELITM SSO HLA Test instructions.

Each strip was identified using a waterproof pen. Generally, 30 strips were hybridized for each run. For each test a positive control (DNA from a lymphoblastoid cell in a Tris-HCl, EDTA solution containing 0.05% sodium azide with a known HLA pattern, supplied with the kit) and a negative control (consisting in bi-distilled water) were included.
The working hybridization buffer (“SSPE concentrate”: Sodium phosphate solution with NaCl, EDTA and 1.0% Proclin® 150 as a preservative and “SDS Concentrate”: Sodium dodecyl sulphate solution with 1% ProClin® 150 diluted in deionized water), the working wash buffer (containing the same reagents at a lower concentration) and the working citrate buffer (a sodium citrate solution diluted in deionized water) were prepared according to manufacturer instructions, using deionized water. The buffers were stored at room temperature.

Working conjugate (Streptavidin-horseradish peroxidase conjugate in a ACES solution with NaCl and 1% ProClin® 150) and substrate solutions (“Substrate A” a citrate solution containing 0.01% H₂O₂ and 0.1% ProClin® 150 and “Substrate B”, consisting in 0.1% 3,3′,5,5′-tetramethylbenzidine (TMB) in 40% dimethylformamide) were always freshly prepared: 15 minutes before use for the conjugate solution and up to 3 hours before use for the substrate solution. The substrate solution was protected from light.

The volumes for each reagent were calculated according to the number of strips in the run.

Amplicons (10 µl of the amplified product) were chemically denatured by adding 10 µl of the “Denaturation Solution” (a solution containing 3% EDTA, 1.6% sodium hydroxide and thymol blue) to each tube and incubating for at least 10 minutes at room temperature. Denaturation results in single strand DNA.

The hybridisation process that follows consists basically in five steps. First, the strips containing a nylon membrane with the immobilized sequence-specific oligonucleotide probes are put in contact with the amplicons, in the presence of the hybridization solution. The amplicons bind (hybridize) to the probes and are captured onto the membrane strips. Secondly, the strips are washed with the wash buffer to remove unbound material. Thirdly, Streptavidin-HRP conjugate is added to each well and binds to the biotin of
the amplified DNA. Fourthly, two new washes are performed with the wash buffer followed by one with the citrate wash to remove unbound conjugate. Finally, the working substrates containing \( \text{H}_2\text{O}_2 \) and TMB are added to react with the bound Streptavidin-HRP conjugate and form a blue colour complex. Membrane strips are then dried using a hot air blower and affixed to the Score sheet.

### 9.5.2.4 Interpretation of results

Validation of the assay was obtained by checking the intensity of the internal control probes. The control probes are designed to give a signal weaker than all other probes in the strip. If signal from the control probes was absent, the assay was invalidated. Any probe signals of equal or stronger intensity of the control probe were scored positive.

Assay quality control was obtained by checking the positive and negative controls. Positive DNA control should give positive signals in the adequate lines, corresponding to the known HLA type for each Class 1 molecule, i.e HLA-A*0101 and A*2301, HLA-B*0801 and B*1801 or B*0801 and B*1803 and HLA-Cw*07011. Negative controls should give no signal. In case of abnormal positive or negative control readings the assay was invalidated.

Hybridized strips were affixed to the Score sheet for scoring positive probes (provided with the typing kit), modified to include a scale to determine probe position for each strip. The pattern for the positive probes was then manually recorded on the Score sheet. To obtain the final HLA type, patterns were interpreted using the Dynal RELI SSO Pattern Matching Program, version 5.11. This software interprets allele distribution according to WHO Nomenclature Committee and International Immunogenetics Information System/HLA database, which is updated twice a year (http://imgt.cines.fr) (Lefranc, 2003).
9.5.3 Typing for Class II molecules

9.5.3.1 Typing method

Typing for Class II molecules consisted in the determination of the HLA-DRB1 category and was performed using the INNO-LiPA HLA-DRB1 Amplification and Hybridization test (Innogenetics, Ghent, Belgium). This assay detects alleles of HLA-DRB1 usually at a two digit resolution level. For certain allelic combinations resolution at the four digit level may be possible. The target sequence for the primers is exon 2 of the DRB1 alleles. The strips contain 37 sequence specific oligoprobes (to define 72 DRB1 alleles) and 2 internal control probes.

All pre-PCR and post-PCR amplification procedures were performed using micropipettes with plugged (aerosol barrier) tips.

9.5.3.2 PCR Amplification reaction

The purified genomic DNA (2.5 μl) was diluted in distilled and sterilized water to obtain a concentration of between 0.01 and 0.02 μg/μl of DNA, was pipetted into PCR tubes (AB gene® Advanced Technologies Ltd, Epsom, Surrey, U.K.) together with 5 μl the amplification buffer (containing all deoxynucleotide triphosphates and 0.05% sodium azide), 5 μl of the DRB1 primer solution (containing biotinylated primers, MgCl₂ and 0.05% sodium azide) and 12 μl of distilled water. Based on previous experience with this type of commercial assay, 5 units of Taq polymerase (Platinum® Taq DNA polymerase, Invitrogen Corp, U.S.A.) were added to each tube. A positive control (LiPA control sample for INNO-LiPA HLA-DRB1) and a negative control (5 μl of distilled water) were used for each amplification reaction.

The samples were placed in a Perkin Elmer Model 9600 thermacycler with a sequence of 95°C for 5 min (denaturation); 35 cycles at 95°C for 20 seconds (denaturation), 58°C for 20 seconds (annealing), and 72°C for 30 seconds (extension); and 10 minutes at 72°C (elongation).
Control of the PCR reaction was performed as for Class I antigens by gel electrophoresis, adding 10 µl of the amplification product per well. The existence of the correct molecular weights was checked as for Class I antigens.

9.5.3.3 Hybridization reaction

Hybridization was performed using an AutoLI PA (Innogenetics, Ghent, Belgium) automated assay processor, programmed to fit the INNO-Li PA HLA-DRB1 hybridisation test.

Each INNO-Li PA HLA-DRB1 strip was identified using a pencil. The volumes for each reagent were calculated according to the number of strips in the run. Generally, 30 strips were hybridized for each run. For each test we included a positive control (Li PA control sample for INNO-Li PA HLA-DRB1, yielding a known pattern of positive probes) and a negative control (consisting in bi-distilled water).

The “Denaturation Solution”, “Hybridisation solution”, “Stringent wash solution”, “Conjugate diluent”, “Conjugate”, “Substrate buffer”, “Substrate BCIP/NBT” and “Rinse solution” were handled according to the manufacturer indications.

Amplicons (5 µl of the solution) were denatured by adding by 5 µl of the “Denaturation Solution” (an alkaline solution containing EDTA). The hybridization solution containing SSPE and 0.5% SDS allowed amplicons to hybridize with the oligonucleotide probes in the strips. Mismatched amplified material was removed by the stringent wash solution (SSPE buffer containing 0.1% SDS). Streptavidin labeled with alkaline phosphatase (in Tris buffer containing protein stabilizers and 0.01% MIT/0.098% CAA as preservative) was added and binds to the biotinylated hybrid. A chromogen-marked substrate solution consisting in a 5-bromo-4-chloro-3-indolyl phosphate-p-toluidine salt (BCIP) and 4-nitroblue tetrazolium (NBT) in dimethylformamide (DMF) was then added to react with the Streptavidin,
resulting in a purple/brown precipitate. The reaction was stopped by a rinse solution (Phosphate buffer containing NaCl, Triton®, 0.05% MIT/0.48% CAA as preservative).

9.5.3.4 Interpretation of results
Validation of the assay was obtained by checking the intensity of the internal control probes. Assay quality control was obtained by checking the positive and negative controls.

Hybridized strips were affixed to the Score sheet for scoring positive probes (provided with the typing kit), modified to include a scale to determine probe position for each strip. The pattern for the positive probes was then manually recorded on the Score sheet.

Interpretation was performed using the LiPA interpretation software LiRAS™. The latest available version, obtained from Abbott, was used.

9.6 STATISTICAL ANALYSIS
9.6.1 Clinical data
Clinical data (demographic characteristics, symptoms and signs, laboratory results) among ES cases and controls were compared using Pearson’s X² or Fisher’s test. Risk factors for ES and death from ES were assessed by calculating the corresponding Odds Ratio (OR) and the 95% Confidence Interval (CI). The Mann-Whitney test was used to compare medians for numeric variables. Standard residuals were evaluated when the variable was not binomial. The statistical software SPSS was used for all calculations and the majority of tables and graphs. MS Excel was also used for data sorting, tables and graphs.

9.6.2 HLA type
Since family data was not available, following allele determination, allele frequencies were compared among cases and controls by determining the p
value of the $X^2$ test applied to a 2 x 2 contingency table. OR and CI were obtained for alleles showing a significant $p$ value.

Haplotypic frequencies were calculated assuming that the two allele loci are in linkage disequilibrium (LD). LD parameter $\Delta$ was obtained by constructing a 2x2 contingency table and applying the formula originally described by Bodmer & Bodmer. LD $\Delta$ was then added to the product of allele frequencies. This approach gives a reliable estimate of a haplotypic frequency with the exception of very small haplotypic frequencies (Schipper, D’Amato et al., 1998).

Significant differences in haplotypic frequency in cases and controls were subsequently obtained using the $X^2$ test with Fisher’s exact test, with the OR and CI. All HLA data analysis was performed in MS Excel, SPSS and EpiInfo.
10 RESULTS

10.1 STUDY POPULATION

Case Report Forms (CRF) for 76 potential ES cases were originally received. 5 patients reported as cases did not meet inclusion criteria: 4 patients with the psychic form of ES, in whom careful revision of the anamnesis showed that those changes were already intermittently present before treatment and one case who developed a single convulsive episode during treatment, in whom a convulsive episode had been observed after the initial lumbar puncture but misreported. Too much data was missing or incorrectly inserted in 3 CRFs of patients reported as ES and they were also refused. After checking for inclusion and exclusion criteria, 69 ES cases and 207 control patients were accepted and included for analysis. The source of included patients is shown in figure 16. Althought none of the selected centres reported unusual incidence rates of ES, the CNPP historically has a high mortality rate, associated with the advanced phase of the HAT patients referred to this centre.

![Pie Chart]

Figure 16: Number of included ES patients according to HAT treatment Centres.

All patients were treated according to the abridged 10 day melarsoprol schedule (IMPAMEL).
The diverse ES preventive interventions (“patient preparation”) used in the centres are described in tables 13, 14 and 15. Mebendazol (200 mg/day), chloroquine (30 mg/kg total dose) were given during 3 days; 3 tablets in single dosage of Fansidar® (sulfadoxine-pyrimethamine) were administered for adults. Quinine was administered for 7 days. Quinine was used as routine only at the CNPP. Supplementation was started before melarsoprol and usually maintained during its administration; prednisone was used usually at 1 mg/kg/day for the duration of the melarsoprol scheme and gradually withdrawn at the end of therapy; hydrocortisone was used at various dosages (100 to 300 mg/day). Promethazine (25 mg/day) was maintained for the duration of the melarsoprol scheme.

<table>
<thead>
<tr>
<th>Antimalarials</th>
<th>Anthelminthic</th>
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</thead>
<tbody>
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</tr>
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</tr>
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<tr>
<td>Maluku</td>
<td>no</td>
</tr>
<tr>
<td>Uíge</td>
<td>yes</td>
</tr>
<tr>
<td>N’Dalatando</td>
<td>yes</td>
</tr>
<tr>
<td>CNPP</td>
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</tr>
</tbody>
</table>

Table 13: Interventions for prevention of ES: antiparasitic drugs

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<th>Corticosteroid</th>
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<th>Antipyretic</th>
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</thead>
<tbody>
<tr>
<td>Prednisone</td>
<td>Hydrocortisone</td>
<td>Promethazine</td>
</tr>
<tr>
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<tr>
<td>M’Buji Mayi</td>
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<tr>
<td>Maluku</td>
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<tr>
<td>Uíge</td>
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<td>yes</td>
</tr>
<tr>
<td>N’Dalatando</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>CNPP</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 14: Interventions for prevention of ES: corticosteroids, antihistaminic, antipyretics. (‘): only 5 out of 32 patients received prednisone due to a shortage in supply. ASA: acetylsalicylic acid.
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<thead>
<tr>
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<th>Multivitamins</th>
<th>Nutritional</th>
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</thead>
<tbody>
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<td>no</td>
</tr>
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<td>no</td>
</tr>
<tr>
<td>Ulge</td>
<td>no</td>
<td>no</td>
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</tr>
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<td>no</td>
</tr>
</tbody>
</table>

Table 15: Interventions for prevention of ES: additional measures used.

10.2 CLINICAL DATA OF THE ENCEPHALOPATHIC SYNDROME CASES

10.2.1 Patient characteristics on admission

Age on admission ranged from 4 to 67 years, with median and mean values of 26 and 30.6 years, respectively (std. deviation: 14.6 years). 3 patients were less than 10 years old and 2 patients were above 60 years. Age distribution is shown in figure 17.
The demographic and epidemiological characteristics of ES cases on admission are shown in table 16.

<table>
<thead>
<tr>
<th>Epidemiological data Parameter</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female/Male</td>
<td>30 / 39</td>
</tr>
<tr>
<td>Diagnostic screening mode</td>
<td>Active/Passive</td>
<td>09 / 60</td>
</tr>
<tr>
<td>Previous treatment</td>
<td>05</td>
<td>7.2</td>
</tr>
<tr>
<td>Alcohol drinkers</td>
<td>15</td>
<td>21.7</td>
</tr>
<tr>
<td>Tobacco smokers</td>
<td>10</td>
<td>14.5</td>
</tr>
<tr>
<td>Hemp smokers</td>
<td>02</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Table 16: Epidemiological data on admission in ES cases. Pentamidine was used in previous treatment in all cases.
The duration of the main complaint (motivating admission) to the treatment centre was available for 62 ES cases. The mean and median duration of complaints were 3.8 and 3 months respectively (range: less than 1 month to 12 months). The temporal distribution of the duration of the main complaint on admission is shown in figure 18.

![Duration of complaint in months](image)

**Figure 18:** Distribution of the duration of the main complaint motivating admission in ES cases, in months. Frequency is in number of patients.

The most often observed main complaints (motivating the consultation) consisted in: change in sleep pattern (reported in the majority of cases as diurnal somnolence) reported isolated by 25 (36.2%) patients and combined with other main complaints by 32 patients (46.4%); headache reported isolated by 15 (21.7%) patients and in combination in 25 (36.2%) patients; and fever reported isolated in 3 (4.3%) patients and in combination in 9 (13%) patients.
Previous diseases reported by ES patients on admission included malaria (21 cases), typhoid fever (4 cases), intestinal parasitosis (4 cases), tuberculosis (2 cases), urinary schistosomiasis (1 case) and syphilis (1 case).

The frequency of symptoms and signs reported in the medical anamnysis on admission is shown in table 17.

The findings in physical examination are shown in table 18. Examination was always performed by the local investigator. Body mass index (BMI), defined as weight (kg) / height $^2$ (cm), was available from 66 patients. BMI mean and median values were 19 (std. deviation 2.9). Tachycardia was defined as pulse equal or more than 100 b.p.m. (excluding patients below 13 years of age). Hypotension was defined as systolic blood pressure equal or less than 100 mmHg (excludes 4 patients below 13 years of age). Fever defined as axillary temperature equal or more than 38°C; all 4 patients with fever had 38°C. Except for the signs indicated in the table, physical examination was generally unremarkable. Skin lesions are described in 29 % of the patients and were mainly not related to HAT (old lesions, fungal infection) but could also be a consequence of pruritus. All other abnormalities in physical examination were present in only one or two patients.
<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms or signs reported in anamnesis</td>
<td>N: 69</td>
</tr>
<tr>
<td><strong>Constitutional signs</strong></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>63</td>
</tr>
<tr>
<td>Fever</td>
<td>49</td>
</tr>
<tr>
<td>Adenomegaly</td>
<td>43</td>
</tr>
<tr>
<td>Inactivity</td>
<td>34</td>
</tr>
<tr>
<td>Anorexia</td>
<td>25</td>
</tr>
<tr>
<td>Pruritus</td>
<td>24</td>
</tr>
<tr>
<td>Dizziness</td>
<td>23</td>
</tr>
<tr>
<td>Weight loss</td>
<td>15</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>13</td>
</tr>
<tr>
<td>Edema</td>
<td>5</td>
</tr>
<tr>
<td><strong>Neuropsychiatric system</strong></td>
<td></td>
</tr>
<tr>
<td>Tremors</td>
<td>28</td>
</tr>
<tr>
<td>Nocturnal insomnia</td>
<td>27</td>
</tr>
<tr>
<td>Walking disability</td>
<td>26</td>
</tr>
<tr>
<td>Motor weakness</td>
<td>23</td>
</tr>
<tr>
<td>Unusual behaviour</td>
<td>21</td>
</tr>
<tr>
<td>Speech impairment</td>
<td>20</td>
</tr>
<tr>
<td>Daytime sleep</td>
<td>20</td>
</tr>
<tr>
<td>Paesthesias</td>
<td>19</td>
</tr>
<tr>
<td>Hyperesthesia</td>
<td>11</td>
</tr>
<tr>
<td>Visual disturbances</td>
<td>8</td>
</tr>
<tr>
<td>Aggressivity</td>
<td>2</td>
</tr>
<tr>
<td><strong>Cardiorespiratory system</strong></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>17</td>
</tr>
<tr>
<td>Thoracic pain</td>
<td>17</td>
</tr>
<tr>
<td>Palpitations</td>
<td>13</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>10</td>
</tr>
<tr>
<td>Precordial pain</td>
<td>8</td>
</tr>
<tr>
<td><strong>Endocrine and genitourinary system</strong></td>
<td></td>
</tr>
<tr>
<td>Amenorrhoea</td>
<td>14 (30)</td>
</tr>
<tr>
<td>Impotence</td>
<td>14 (39)</td>
</tr>
<tr>
<td>Low urine output</td>
<td>15</td>
</tr>
<tr>
<td>Abortion</td>
<td>04 (30)</td>
</tr>
<tr>
<td>Polyuria/polakuria</td>
<td>9</td>
</tr>
<tr>
<td>Disuria</td>
<td>7</td>
</tr>
<tr>
<td>Infertility</td>
<td>7</td>
</tr>
<tr>
<td>Hematuria</td>
<td>1</td>
</tr>
<tr>
<td><strong>Osteomuscular system</strong></td>
<td></td>
</tr>
<tr>
<td>Back pain</td>
<td>30</td>
</tr>
<tr>
<td>Myalgia</td>
<td>29</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>28</td>
</tr>
<tr>
<td>Bone pain</td>
<td>21</td>
</tr>
<tr>
<td><strong>Digestive system</strong></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>21</td>
</tr>
<tr>
<td>Obstipation</td>
<td>20</td>
</tr>
<tr>
<td>Vomiting</td>
<td>10</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 17: Symptoms and signs reported in the anamnesis. Numbers refer to 69 patients except for gender-related variables, where the number of patients is indicated in brackets.
<table>
<thead>
<tr>
<th>Clinical data Physical examination</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td><strong>Percentage</strong></td>
</tr>
<tr>
<td>General status Bad / Fair / Good</td>
<td>3 / 41 / 25</td>
</tr>
<tr>
<td><strong>Body mass index</strong></td>
<td></td>
</tr>
<tr>
<td>More than 20</td>
<td>24 (66)</td>
</tr>
<tr>
<td>Between 18 and 20</td>
<td>24 (66)</td>
</tr>
<tr>
<td>Less than 18</td>
<td>18 (66)</td>
</tr>
<tr>
<td>Lymph node enlargement</td>
<td>42</td>
</tr>
<tr>
<td>Hypotension</td>
<td>38 (65)</td>
</tr>
<tr>
<td>Cheiro-oral primitive reflex</td>
<td>21</td>
</tr>
<tr>
<td>Tremors</td>
<td>20</td>
</tr>
<tr>
<td>Apathy</td>
<td>18</td>
</tr>
<tr>
<td>Abnormal gait</td>
<td>17</td>
</tr>
<tr>
<td>Decreased muscular tonus</td>
<td>16</td>
</tr>
<tr>
<td>Slurred or incomprehensible speech</td>
<td>15</td>
</tr>
<tr>
<td>Depression</td>
<td>13</td>
</tr>
<tr>
<td>Peri-oral primitive reflex</td>
<td>12</td>
</tr>
<tr>
<td>Romberg sign</td>
<td>11</td>
</tr>
<tr>
<td>Decreased muscular strength</td>
<td>15</td>
</tr>
<tr>
<td>Confusional status</td>
<td>07</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>06 (65)</td>
</tr>
<tr>
<td>Fever</td>
<td>04</td>
</tr>
</tbody>
</table>

Table 18: Signs and symptoms observed on admission during physical examination. The number of evaluated patients is 69 except when otherwise indicated in brackets.

Laboratory evaluation on admission of ES patients is shown in table 19 and 20. All patients had confirmed parasitological diagnosis, except patients with previous treatment with pentamidine, who were diagnosed according to a positive CATT and an increased CSF WBC count. 8 CSF samples were obtained by the mobile team (WBC count: mean 82.75; median: 37.5; range: 8 to 312) and were not repeated at the treatment Centre. Additional tests performed include urine analysis in two patients. Stool analysis was not performed. No other tests (including HIV testing) were performed.
### Clinical data

#### Parasitological diagnosis

<table>
<thead>
<tr>
<th>Parasitological diagnosis</th>
<th>Positive/Tested</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanosomes in lymph</td>
<td>41 / 49</td>
<td>83.7</td>
</tr>
<tr>
<td>Trypanosomes in blood</td>
<td>09 / 33</td>
<td>27.3</td>
</tr>
<tr>
<td>Trypanosomes in CSF</td>
<td>44 / 61</td>
<td>73.3</td>
</tr>
<tr>
<td>WBC count in CSF (n° cells/mm³)</td>
<td>61/61</td>
<td>100</td>
</tr>
</tbody>
</table>

#### Additional laboratory data

<table>
<thead>
<tr>
<th>Hemoglobin (mg/dL)</th>
<th>Tested / (%)</th>
<th>Central tendency measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>17 (24.6)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>WBC in blood (x10^3/μL)</td>
<td>12 (17.4)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8170</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>7850</td>
<td></td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>3581</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>4000 – 15600</td>
<td></td>
</tr>
</tbody>
</table>

### 10.2.2 Characteristics of the encephalopathic syndrome

#### 10.2.2.1 Clinical features

The more frequently present symptoms and signs in ES cases and corresponding degree are shown in the table 21. Degree 1 is defined as transient, present, mild, or localized. Degree 2 is defined as durable and needing intervention, intolerable, severe, or diffuse. For fever, degree 1 is defined as 37.5 - 38.9 ºC, degree 2 as more than 39ºC. For tachycardia, degree 1 is defined as less than 100 bpm, degree 2 as more than 100 bpm. For
hypotension, degree 1 is defined as systolic blood pressure less than 80 mmHg, degree 2 as shock. Degree 2 precordial pain, diarrhea, and bullous eruption were observed in two cases each. Jaundice was not observed. Fundoscopic examination was not performed in ES patients.

<table>
<thead>
<tr>
<th>Characterization of ES Symptoms and signs</th>
<th>Frequency (Global)</th>
<th>Frequency (Degree 1)</th>
<th>Frequency (Degree 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaise</td>
<td>79.7 (55)</td>
<td>20.3</td>
<td>59.4</td>
</tr>
<tr>
<td>Confusional state</td>
<td>78.3 (51)</td>
<td>26.1</td>
<td>52.2</td>
</tr>
<tr>
<td>Fever</td>
<td>69.6 (48)</td>
<td>52.2</td>
<td>17.4</td>
</tr>
<tr>
<td>Agitation</td>
<td>65.2 (45)</td>
<td>34.8</td>
<td>30.4</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>60.9 (42)</td>
<td>39.1</td>
<td>21.7</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td>59.4 (41)</td>
<td>36.2</td>
<td>23.2</td>
</tr>
<tr>
<td>Headache</td>
<td>56.5 (39)</td>
<td>37.7</td>
<td>18.8</td>
</tr>
<tr>
<td>Apathy</td>
<td>56.5 (39)</td>
<td>24.6</td>
<td>31.9</td>
</tr>
<tr>
<td>Maculopapular eruption</td>
<td>50.7 (35)</td>
<td>10.1</td>
<td>40.6</td>
</tr>
<tr>
<td>Chills</td>
<td>36.2 (25)</td>
<td>24.6</td>
<td>11.6</td>
</tr>
<tr>
<td>Red eye syndrome</td>
<td>33.3 (23)</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>Vertigo</td>
<td>29.0 (20)</td>
<td>20.3</td>
<td>8.7</td>
</tr>
<tr>
<td>Edema (facial)</td>
<td>23.2 (16)</td>
<td>23.2</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>23.2 (16)</td>
<td>14.5</td>
<td>8.7</td>
</tr>
<tr>
<td>Babinsky sign</td>
<td>20.3 (14)</td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td>Panic attack</td>
<td>18.8 (13)</td>
<td>14.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Nausea</td>
<td>18.8 (13)</td>
<td>13.0</td>
<td>5.8</td>
</tr>
<tr>
<td>Hypotension</td>
<td>15.9 (11)</td>
<td>13.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>10.1 (07)</td>
<td>8.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Delirium</td>
<td>10.1 (07)</td>
<td>0</td>
<td>10.1</td>
</tr>
<tr>
<td>Nucal rigidity</td>
<td>10.1 (07)</td>
<td>10.1</td>
<td></td>
</tr>
<tr>
<td>Aggressive behaviour</td>
<td>07.2 (05)</td>
<td>4.3</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Table 21: Symptoms and signs characterizing ES, according to the global frequency and discriminated for the degree of severity.
The type of manifestation of ES observed and the corresponding outcome is shown in table 22. Three categories could be determined: convulsions followed by coma; convulsions without coma; and coma without convulsions. Convulsions were present in 85.4% of the patients. Convulsions were usually multiple but 24.6% of the cases had one single convulsive episode.

For coma characterization we used a modified Glasgow Score (see CRF), where unarousable coma is associated with a score of 7 or less. During the ES episode, the mean minimal score was 4.83, with a median score of 5 (Std. Deviation: 2.6) and the mean maximal score was 6.29, with a median score of 6 (Std. Deviation: 2.5). The exclusively mental type of ES was not observed.
<table>
<thead>
<tr>
<th>Type of manifestation of ES</th>
<th>Cases N / (%)</th>
<th>Outcome survival n / %</th>
<th>Additional signs n</th>
<th>Outcome death n / %</th>
<th>Additional Signs n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Convulsions (multiple episodes) and coma</strong></td>
<td>26 / (37.7)</td>
<td>5 / (7.2)</td>
<td>No additional signs</td>
<td>21 / (30.4)</td>
<td>Fever in 13 Urticaria in 7 Fever and urticaria in 5</td>
</tr>
<tr>
<td><strong>Convulsion (single episode) and coma</strong></td>
<td>12 / (17.4)</td>
<td>8 / (11.6)</td>
<td>Fever in 3 Urticaria in 3 Fever and urticaria in 1</td>
<td>4 / (5.8)</td>
<td>Fever in 2</td>
</tr>
<tr>
<td><strong>Convulsion and coma Sub-totals</strong></td>
<td>38 / (55)</td>
<td>13 / (18.8)</td>
<td>Fever in 3 Urticaria in 3 Fever and urticaria in 1</td>
<td>25 / (36.2)</td>
<td>Fever in 15 Urticaria in 7 Fever and urticaria in 5</td>
</tr>
<tr>
<td><strong>Convulsions multiple (episodes) without coma</strong></td>
<td>16 / (23.2)</td>
<td>14 / (20.3)</td>
<td>Fever in 3 Fever and urticaria in 1</td>
<td>2 / (2.9)</td>
<td>Fever in 1</td>
</tr>
<tr>
<td><strong>Convulsion (single episode) without coma</strong></td>
<td>5 / (7.2)</td>
<td>5 / (7.2)</td>
<td>Fever in 3 Urticaria in 4 Fever and urticaria in 1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Convulsion without coma Sub-totals</strong></td>
<td>21 / (30.4)</td>
<td>19 / (27.5)</td>
<td>Fever in 6 Urticaria in 4 Fever and urticaria in 2</td>
<td>2 / (2.9)</td>
<td>Fever in 1</td>
</tr>
<tr>
<td><strong>Coma without convulsions</strong></td>
<td>10 / (14.5)</td>
<td>5 / (7.2)</td>
<td>Fever in 2 Fever and urticaria in 1</td>
<td>5 / (7.2)</td>
<td>Fever in 2 Urticaria in 1</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>69 / (100)</td>
<td>37 / (53.6)</td>
<td>32 / (46.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 22: Type of manifestation of ES observed and discriminated according to the outcome. The existence of fever and/or maculopapular cutaneous eruption (urticaria) is also indicated.
10.2.2.2 Time point for ES

The mean and median numbers of melarsoprol applications associated with the development of ES were 8.52 and 9 doses, respectively (range: 2 to 10 doses; Std. Deviation: 1.9). Melarsoprol was administered on consecutive days in all cases, except in one hypertensive patient due to hypertension peaks. 47.8 % of patients developed ES with melarsoprol 10th dose. Figure 19 shows the distribution of ES according to the number of doses of melarsoprol received.

![Figure 19: Occurrence of ES following the initial application of melarsoprol, according to the number of doses received. Frequency is in number of patients.](image)

Figure 20 shows the distribution of the two types of initial manifestation of ES (coma or convulsion) according to the doses of melarsoprol received. The difference between the number of doses of melarsoprol needed to trigger coma or convulsions is not significantly different between the two groups (p value of the Mann-Whitney test: 0.26; all observed counts not statistically different from those expected: all std. residuals below 1.96).
The mean time interval between the last application of melarsoprol and the development of ES was 35.44 hours. Half of the patients developed ES within 23.5 hours after the last application and 75% did so 47.65 hours within the last drug application. Table 23 and figure 21 show the time interval distribution for development of ES after the last melarsoprol application, discriminated according to the final outcome of ES. Although there is a trend towards a more rapid occurrence of ES in patients with a good outcome, the p value for the Mann-Whitney test comparing the time for development of ES in survivors and fatalities is not significant (0.08).
<table>
<thead>
<tr>
<th>Central tendency measures</th>
<th>Time for ES in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N: 37</td>
</tr>
<tr>
<td>Survival</td>
<td>Death</td>
</tr>
<tr>
<td>Mean</td>
<td>30.9</td>
</tr>
<tr>
<td>Median</td>
<td>12.75</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>41.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>181.5</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 23: Time in hours for the development of ES after the last melarsoprol application, discriminated according to the final outcome of ES.

Figure 21: Time interval in hours for the occurrence of ES after the last melarsoprol application according to the final outcome. The inbox line in bold indicates the median. Outliers and extremes are not shown.
10.2.2.3 Laboratory evaluation of ES

15 patients were evaluated within the duration of the ES regarding hemoglobin, and leukocyte count in blood and in CSF. A Gram smear was performed in 14 CSF samples, always with a negative result. In 11 patients, CSF glucose content was also obtained but was not simultaneously measured in blood. Protein content of CSF was obtained only in two cases at the CNPP (values: 60 and 90 mg/dL). The *Plasmodium* smear was negative in all ES cases evaluated for CSF parameters. The results of the laboratory evaluation of ES are shown in table 24.

<table>
<thead>
<tr>
<th>Laboratory evaluation of ES</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tested n (%)</td>
</tr>
<tr>
<td>Hemoglobin (mg/dL)</td>
<td>15 (21.7)</td>
</tr>
<tr>
<td>Mean</td>
<td>10.6</td>
</tr>
<tr>
<td>Median</td>
<td>10.7</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.96</td>
</tr>
<tr>
<td>Range</td>
<td>7.15</td>
</tr>
<tr>
<td>WBC in blood (x10^3/µL)</td>
<td>15 (21.7)</td>
</tr>
<tr>
<td>Mean</td>
<td>6071</td>
</tr>
<tr>
<td>Median</td>
<td>4500</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>2353</td>
</tr>
<tr>
<td>Range</td>
<td>4000 – 11800</td>
</tr>
<tr>
<td>WBC count in CSF (n°cells/mm^3)</td>
<td>15 (21.7)</td>
</tr>
<tr>
<td>Mean</td>
<td>46.1</td>
</tr>
<tr>
<td>Median</td>
<td>24</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>51.5</td>
</tr>
<tr>
<td>Range</td>
<td>2 – 176</td>
</tr>
<tr>
<td>CSF glucose content (mg/dL)</td>
<td>11 (15.9)</td>
</tr>
<tr>
<td>Mean</td>
<td>54.9</td>
</tr>
<tr>
<td>Median</td>
<td>51</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>23.8</td>
</tr>
<tr>
<td>Range</td>
<td>21 – 117</td>
</tr>
</tbody>
</table>

Table 24: Laboratory evaluation of ES patients.
10.2.2.4 Concomitant diseases

Additional diagnosis on admission in ES cases included oral candidosis, lymphatic filariasis, pulmonary tuberculosis and orchitis in one patient each. Only the patient with oral candidosis did not develop ES. The three other patients died from ES.

During ES, a clinical diagnosis of respiratory infection was clearly established in 2 patients and one died.

The situation regarding malaria (defined as a positive thick smear) on admission or during ES and corresponding outcome of ES is complex and is described as follows (table 25):

- 35 patients were initially not tested for *Plasmodium* (thick smear) on admission. Subsequently, 9 out of 34 patients tested during ES were positive for *Plasmodium* and 6 died from ES. 25 of those were negative for *Plasmodium* and 11 died from ES.

- 34 patients were tested for *Plasmodium* on admission. Of these, 25 were negative. 24 were again tested during ES: 4 showed a positive smear and 2 died from ES. 20 patients remained negative for *Plasmodium* and 12 died from ES.
<table>
<thead>
<tr>
<th>On admission</th>
<th>During ES</th>
<th>Death from ES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tested 35</td>
<td>Tested 34</td>
</tr>
<tr>
<td>Not tested 35</td>
<td>Tested 34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive 09</td>
<td>03 (18.75)</td>
</tr>
<tr>
<td></td>
<td>Negative 25</td>
<td>14 (34.37)</td>
</tr>
<tr>
<td>Tested 34</td>
<td>Tested 32</td>
<td></td>
</tr>
<tr>
<td>Negative 25</td>
<td>Positive 04</td>
<td>02 (6.25)</td>
</tr>
<tr>
<td></td>
<td>Negative 20</td>
<td>08 (37.5)</td>
</tr>
<tr>
<td>Positive 09</td>
<td>Positive 06</td>
<td>05 (3.12)</td>
</tr>
<tr>
<td></td>
<td>Negative 02</td>
<td>00 (00)</td>
</tr>
</tbody>
</table>

Table 25: Concomitant malaria, defined as a positive thick smear, according to the initial test on admission and during ES and in relation to the outcome of ES. Plasmodium trophozoite count ranged from 1 to 3 per field.

### 10.2.3 Management of the encephalopathic syndrome

The principal interventions aimed at treating ES and corresponding outcome are shown in table 26. Patients received several combinations of the drugs shown, depending on the clinical picture and on the therapeutic approach chosen by the attending clinician. Two patients did not receive corticosteroids and they both survived. Intravenous fluids were used in 61 patients, consisting basically of 5% glucose in water or Ringer lactate when available. A urethral catheter was inserted in 40 patients and a nasogastric tube was used in 13. No evident anomalies in drug use, namely overdosage, were detected.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of patients</th>
<th>Death from ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td>54</td>
<td>27</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>13</td>
<td>05</td>
</tr>
<tr>
<td>Diazepam and phenobarbital</td>
<td>29</td>
<td>09</td>
</tr>
<tr>
<td>Furosemide</td>
<td>08</td>
<td>07</td>
</tr>
<tr>
<td>Promethazine</td>
<td>08</td>
<td>03</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>08</td>
<td>05</td>
</tr>
<tr>
<td>ASA</td>
<td>08</td>
<td>04</td>
</tr>
<tr>
<td>Quinine</td>
<td>07</td>
<td>05</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>05</td>
<td>04</td>
</tr>
<tr>
<td>Mannitol</td>
<td>05</td>
<td>04</td>
</tr>
</tbody>
</table>

Table 26: Drugs used in ES management and outcome.

10.2.4 Outcome of ES

The distribution of fatalities due to ES by treatment centre is shown in figure 22.

Figure 22: Distribution of fatalities in the treatment centres. The total number of fatalities and the percentage of ES cases dying are indicated.
The final outcome of ES was defined in average 2.4 days after diagnosis (median: 2 days; std. deviation: 2.3 days; range 0 -13 days). The central tendency measures for the duration of ES according to the final outcome of ES are shown in table 27.

Death was the final outcome of ES in 32 patients (46.4 %). The fatal outcome was defined in average 2.5 days after diagnosis (median: 1 day; std. deviation: 2.9 days; range 0 -13 days). 58.1% and 80.6% of the patients died within one day and 3 days after developing ES, respectively. Figure 23 shows the number of fatalities according to the time frame for ES.

<table>
<thead>
<tr>
<th>Central tendency measures</th>
<th>Duration of ES in days</th>
<th>N: 37</th>
<th>N: 32</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survival</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td>1.6</td>
<td>2.9</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 27: Duration of ES after diagnosis, according to the final outcome.
The majority of patients (27 out of 37, 73%) surviving ES did not show any sequelae on discharge. When present, sequelae consisted in tremors (3 cases) mental changes (confusion, euphoria, aggressivity) (3 cases) paraplegia (2 cases, one with urinary retention), speech disturbances (polyphonia, dysphonia, aphasia) (3 cases) and walking disability (1 case).

10.3 **Analysis of Risk Factors**

10.3.1 **Clinical Risk Factors**

All variables in the questionnaire were analysed to determine if they constitute risk factors for the development of ES or for mortality from ES (case fatality rate, CFR). Table 28 shows the variables obtained on admission that yield a significant p value (< 0.05) and selected variables with a higher p value when the OR indicates a tendency, even when the lower limit of the 95% CI is below 1. All other variables yielded not significant p or OR values.
For the numeric variables age and WBC in CSF, the Mann-Whitney test was used to compare medians; standard residuals values while applying the $X^2$ test were checked for values above $> 1.96$. Age and WBC in CSF values were split into quartiles.

Regarding age and the development of ES, the Mann-Whitney test yields a not significant p value of 0.1. Quartiles 25, 50 and 75 % corresponded to age 22, 32 and 41 years, respectively. Cases with age below 22 years showed an observed count higher then the expected (26% versus 18.1%) with a std. residual value of 1.9. More patients were below the median than above the median (32 years), but the p value is not significant (0.1).

For age and death from ES, the Mann-Whitney test yields also a not significant p value of 0.9. Observed frequencies in quartiles were not statistically different from those expected (all std. residual values $< 1.96$) and the number of patients above and below the median (26 years) was similar.

The Mann-Whitney test for WBC in CSF and the development of ES yields a not significant p value of 0.1. 25, 50 and 75 % quartiles correspond to 100, 235, and 410 WBC in CSF, respectively. Observed frequencies in quartiles were not statistically different from those expected (all std. residual values $< 1.96$); the number of patients above the median (235 cells) was greater than those below but the difference is not significant (p: 0.3).

For WBC and death from ES, the p value of the Mann-Whitney test is 0.3. The number of patients above and below the median was similar. Observed frequencies in quartiles were not statistically different from those expected (all std. residual values $< 1.96$) and the number of patients above and below the median (283 cells) was similar.

For evaluation of body mass index (BMI) and general status on admission, proportions were compared by crosstabulation. BMI was defined by the usual
formula. Three categories were defined for BMI corresponding to severe malnutrition (BMI ≤ 18), moderate malnutrition (BMI >18 and ≤ 20) and normal nutritional status (BMI >20). The p values for BMI and general status were not significant. Standard residuals did not show any significant difference.

Variables observed during ES and associated or with some degree of association (indicated by the value of the OR even for not significant p values) with death are shown in table 29.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Encephalopathy</th>
<th></th>
<th></th>
<th>Death</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>p</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Anamnesis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edema</td>
<td>4.0</td>
<td>1.0 - 15.4</td>
<td>0.03</td>
<td>1.8</td>
<td>0.2 - 11.5</td>
</tr>
<tr>
<td>Bone pain</td>
<td>2.3</td>
<td>1.2 - 4.3</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paralysis</td>
<td>3.0</td>
<td>0.4 - 22.0</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoestesia</td>
<td>2.0</td>
<td>0.3 - 12.2</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>4.5</td>
<td>1.5 - 14.0</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>3.0</td>
<td>1.0 - 8.7</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pruritus</td>
<td>3.0</td>
<td>1.0 - 8.7</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenomegaly</td>
<td>2.2</td>
<td>0.7 - 6.1</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>1.9</td>
<td>0.6 - 5.6</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td>2</td>
<td>0.6 - 6.6</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weakness/asthenia</td>
<td>2</td>
<td>0.5 - 7.4</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4.9</td>
<td>0.9 - 25.6</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical examination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal vision</td>
<td>5.9</td>
<td>2.2 - 15.4</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal eye movements</td>
<td>4.9</td>
<td>1.5 - 16.7</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal hearing</td>
<td>6.0</td>
<td>1.4 - 25.8</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apathy</td>
<td>1.9</td>
<td>1.0 - 3.8</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>1.9</td>
<td>0.9 - 4.2</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facial paralysis</td>
<td>3.5</td>
<td>0.6 - 17.8</td>
<td>0.1</td>
<td>0.4</td>
<td>0.3 - 0.6</td>
</tr>
<tr>
<td>Incomprehensible</td>
<td>2.2</td>
<td>0.5 - 7.7</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Speech</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Babinsky</td>
<td>2.2</td>
<td>0.6 - 7.7</td>
<td>0.3</td>
<td>3.6</td>
<td>0.4 - 36.7</td>
</tr>
<tr>
<td>Pale mucosa</td>
<td>2.4</td>
<td>0.2 - 27.7</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neck rigidity</td>
<td>2.4</td>
<td>0.2 - 27.7</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>2.8</td>
<td>0.2 - 33.6</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agitation</td>
<td>2.4</td>
<td>0.2 - 27.7</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Laboratory data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypanosomes in lymph</td>
<td>1.8</td>
<td>0.7 - 4.7</td>
<td>0.2</td>
<td>0.9</td>
<td>0.2 - 3.9</td>
</tr>
<tr>
<td>Trypanosomes in CSF</td>
<td>1.2</td>
<td>0.6 - 2.2</td>
<td>0.6</td>
<td>2.2</td>
<td>0.6 - 7.4</td>
</tr>
</tbody>
</table>

Table 28: Anamnesis, physical examination and laboratory variables obtained during admission and corresponding statistical tests for association with the development of ES or death from ES. (*) indicates that the Fisher's test was used.
<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coma</td>
<td>2.4</td>
<td>1.8 – 3.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td>5</td>
<td>1.7 – 14.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Babinsky</td>
<td>3.8</td>
<td>1.0 – 13.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Vertigo</td>
<td>2.2</td>
<td>1.0 - 4.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>2.0</td>
<td>0.7 – 5.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Hypotension</td>
<td>2.4</td>
<td>0.6 – 9.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Agitation</td>
<td>1.7</td>
<td>0.6 – 4.8</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 29: Variables observed during ES and corresponding statistical tests for association with death from ES.

10.3.2 HLA types

Alleles for every tested HLA category could be clearly defined in samples corresponding to 62 ES cases and 189 controls. Samples showing any ambiguity in allele determination were not included in the analysis.

The frequency of alleles in individuals were determined for HLA-DR, HLA-A, HLA-B and HLA-Cw classes. The results of allele determination in cases and controls with respective frequencies are shown in tables 30, 31, 32 and 33. The deducted haplotypes in cases and controls with respective frequencies are shown in table 34. OR and CI are shown for alleles with a p value of at least 0.2. The most significant ORs in each HLA category are highlighted in bold.
<table>
<thead>
<tr>
<th>HLA-DR</th>
<th>Controls</th>
<th>Cases</th>
<th>Cases vs controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>allele</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>01</td>
<td>28</td>
<td>7.4</td>
<td>10</td>
</tr>
<tr>
<td>03</td>
<td>43</td>
<td>11.4</td>
<td>13</td>
</tr>
<tr>
<td>04</td>
<td>10</td>
<td>2.6</td>
<td>00</td>
</tr>
<tr>
<td>07</td>
<td>27</td>
<td>7.1</td>
<td>17</td>
</tr>
<tr>
<td>08</td>
<td>10</td>
<td>2.6</td>
<td>01</td>
</tr>
<tr>
<td>09</td>
<td>11</td>
<td>2.9</td>
<td>06</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>4.8</td>
<td>03</td>
</tr>
<tr>
<td>11</td>
<td>62</td>
<td>16.4</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>4.0</td>
<td>03</td>
</tr>
<tr>
<td>13</td>
<td>65</td>
<td>17.2</td>
<td>29</td>
</tr>
<tr>
<td>14</td>
<td>03</td>
<td>0.8</td>
<td>03</td>
</tr>
<tr>
<td>15</td>
<td>84</td>
<td>22.2</td>
<td>24</td>
</tr>
<tr>
<td>16</td>
<td>02</td>
<td>0.5</td>
<td>01</td>
</tr>
<tr>
<td>Total</td>
<td>378</td>
<td>124</td>
<td></td>
</tr>
</tbody>
</table>

Table 30: HLA-DR allele distribution in controls and cases, with respective p value, OR and 95% CI. Ns: not significant.
<table>
<thead>
<tr>
<th>HLA-A</th>
<th>Controls</th>
<th>Cases</th>
<th>Cases vs controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>allele</td>
<td>N</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>01</td>
<td>08</td>
<td>2.12</td>
<td>05</td>
</tr>
<tr>
<td>02</td>
<td>49</td>
<td>12.96</td>
<td>20</td>
</tr>
<tr>
<td>03</td>
<td>28</td>
<td>7.41</td>
<td>09</td>
</tr>
<tr>
<td>23</td>
<td>61</td>
<td>16.14</td>
<td>24</td>
</tr>
<tr>
<td>24</td>
<td>04</td>
<td>1.06</td>
<td>03</td>
</tr>
<tr>
<td>26</td>
<td>02</td>
<td>0.53</td>
<td>03</td>
</tr>
<tr>
<td>29</td>
<td>20</td>
<td>5.29</td>
<td>03</td>
</tr>
<tr>
<td>30</td>
<td>75</td>
<td>19.84</td>
<td>18</td>
</tr>
<tr>
<td>31</td>
<td>03</td>
<td>0.79</td>
<td>00</td>
</tr>
<tr>
<td>32</td>
<td>02</td>
<td>0.53</td>
<td>03</td>
</tr>
<tr>
<td>33</td>
<td>10</td>
<td>2.65</td>
<td>04</td>
</tr>
<tr>
<td>34</td>
<td>17</td>
<td>4.50</td>
<td>02</td>
</tr>
<tr>
<td>36</td>
<td>19</td>
<td>5.03</td>
<td>04</td>
</tr>
<tr>
<td>66(10)</td>
<td>16</td>
<td>4.23</td>
<td>06</td>
</tr>
<tr>
<td>68(28)</td>
<td>35</td>
<td>9.26</td>
<td>13</td>
</tr>
<tr>
<td>74(19)</td>
<td>26</td>
<td>6.88</td>
<td>06</td>
</tr>
<tr>
<td>80</td>
<td>03</td>
<td>0.79</td>
<td>01</td>
</tr>
<tr>
<td>Total</td>
<td>378</td>
<td>124</td>
<td></td>
</tr>
</tbody>
</table>

Table 31: HLA-A allele distribution in controls and cases, with respective p value, OR and 95% CI. Ns: not significant. (*) indicates Fisher's test was used.
<table>
<thead>
<tr>
<th>HLA-B</th>
<th>Controls</th>
<th>Cases</th>
<th>Cases vs controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>07</td>
<td>27</td>
<td>7.1</td>
<td>12</td>
</tr>
<tr>
<td>08</td>
<td>15</td>
<td>4.0</td>
<td>05</td>
</tr>
<tr>
<td>13</td>
<td>06</td>
<td>1.6</td>
<td>02</td>
</tr>
<tr>
<td>14</td>
<td>28</td>
<td>7.4</td>
<td>11</td>
</tr>
<tr>
<td>15</td>
<td>65</td>
<td>17.2</td>
<td>29</td>
</tr>
<tr>
<td>18</td>
<td>14</td>
<td>3.7</td>
<td>03</td>
</tr>
<tr>
<td>35</td>
<td>22</td>
<td>5.8</td>
<td>06</td>
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<tr>
<td>Total</td>
<td>378</td>
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Table 32: HLA-B allele distribution in controls and cases, with respective p value, OR and 95% CI. Ns: not significant.
<table>
<thead>
<tr>
<th>HLA-C</th>
<th>Controls</th>
<th>Cases</th>
<th>Cases vs controls</th>
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<tr>
<td>allele</td>
<td>n</td>
<td>%</td>
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<tr>
<td>Total</td>
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<td>124</td>
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Table 33: HLA-Cw allele distribution in controls and cases, with respective p value, OR and 95% CI. Ns: not significant. (*) indicates Fisher’s test was used.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Controls</th>
<th>p</th>
<th>OR</th>
<th>CI</th>
<th>LD Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>C<em>14  / B</em>15</td>
<td>6/62</td>
<td>3/189</td>
<td>0.008 (*)</td>
<td>6.64</td>
<td>1.35 - 41.96</td>
</tr>
<tr>
<td>A<em>23  / C</em>14</td>
<td>3/62</td>
<td>1/189</td>
<td>0.04 (*)</td>
<td>9.56</td>
<td>0.74 – 50.4</td>
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<tr>
<td>A<em>23  / B</em>15</td>
<td>13/62</td>
<td>22/189</td>
<td>0.06</td>
<td>2.0</td>
<td>0.88 - 4.56</td>
</tr>
<tr>
<td>DR<em>07 / B</em>58</td>
<td>5/62</td>
<td>5/189</td>
<td>0.07 (*)</td>
<td>3.23</td>
<td>0.71 -14.49</td>
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</table>

Table 34: Frequency of deducted haplotypes in cases and controls, with OR and CI. (*) indicates Fisher’s test was used.
11 DISCUSSION

11.1 STUDY METHODOLOGY PERFORMANCE

The medical questionnaire used in the present study allowed a detailed prospective collection of clinical data in the study population. Given the different educational medical backgrounds and differently developed medical skills observed in the local investigators, presenting anamnesis and physical examination variables in a structured way was critical in data acquisition. The study implementation workshops performed in each country, aimed mainly at clearly establishing definitions and refreshing clinical skills with emphasis on neurology, also contributed for the reasonably good homogeneity and quality of the data obtained. However, data acquisition was naturally depending on each clinician history-taking and physical examination skills and these influenced the quality and the level of detail of the reported observation.

The time needed for CRF data insertion was around 30 - 40 minutes per patient, after the initial difficulties related to the concept of the study and to the complexity of the questionnaire were surmounted. Data quality was increasingly better as the study progressed. Following the monitoring visits and on site discussion of the difficulties encountered, the CRFs retrieved in the second half of the study were generally of good or very good quality. The good motivation of the participating local staff also contributed for reaching 76 of the initially proposed 100 ES cases within the proposed timeframe, even if after checking for the strict inclusion and exclusion study criteria, only 69 ES cases were finally retained as valid. Despite all efforts developed, the difficult working conditions in the field, the complexity of the study and the limited time frame precluded obtaining the established number of 100 ES cases. The insertion of the present study within the IMPAMEL II program framework was fundamental for the success in the collaboration with the ICCT and with the PNLTHA for study implementation and management.
We believe that with adequate training sessions on patient history-taking and physical examination, the CRF developed for the present study, although complex and extensive, may be used as a field tool for clinical and epidemiological studies on sleeping sickness. The form can be easily adapted to different needs and allows the prospective collection of clinical data in a standardized way. An adapted version of this CRF has already been used for clinical data acquisition during the DB 289 clinical trial. Furthermore, the initial part of the questionnaire referring to the medical anamnesis and physical examination is presently under evaluation as the new standard for clinical data collection in HAT centres with a full-time clinician in Angola and in the DRC.

The blood sample collection method using the Gentra Generation® Sample Collection Cards proved to be easy, adequate and convenient for field conditions. After the technique was adapted to the routine and optimized at the Centro de Histocompatibilidade do Norte, DNA for HLA typing could be purified from the cards without major problems, up to more than 12 months after blood collection with storage at room temperature. The amount of blood collected in the cards was generally 6 to 10 times more than the amount needed to perform two DNA purifications with the Gentra method. In practice, the HLA study would not have been possible with a conventional blood sample collection method, since none of the centres was able to separate blood cells (buffy coat) and freeze them in liquid nitrogen, which would have been the alternative for sample collection.

The quality of the DNA purified from the Gentra Generation® Sample Collection Cards was sufficient to allow a correct performance of the two methods used for HLA typing. However, in around 20% of the samples, the DNA purification procedure had to be repeated once or twice until a correct typing result could be obtained. This difficulty was more evident in old Gentra Cards (collected more than 9 month before purification) and was considered to be a consequence of sample contamination. 9% of the total
number of collected samples (7 from ES cases and 18 from controls) showed
ambiguities in allele determination with the methods used and could not be
included in the association study. Clarification of the detected ambiguities
would need high definition typing methods or gene sequencing, which would
have added to the financial cost of the project. Although the use of these
more precise methods would have slightly increased the power of the
association study and could possibly allow the identification of new alleles,
their application felt beyond the scope of the present study.

The statistical methods used in study population characterization and in risk
factor analysis were descriptive and simple. Given the size of the ES
population in the study and the low number of significant risk factors found,
any additional manipulation of data such as stratification or logistic regression
could have brought potentially interesting but insufficiently powered results.
Tendencies are however described whenever they seem to correspond to an
important biological concept.

II.2 CLINICAL CHARACTERISTICS OF THE ENCEPHALOPATHIC SYNDROME

11.2.1 Clinical Picture
The characteristics of the encephalopathic syndrome could be obtained with a
good level of discriminating details.

In 3/4 of the patients ES started within less than 48 hours after the last
preceding melarsoprol dose. Convulsions were present in 85.5% of all cases
and are therefore considered to be the principal characteristic of ES. The
convulsions found in ES are frequently multiple; these were observed in 60%
of all ES cases and constituted 71% of all convulsive episodes. Convulsions
led to coma in 55% of all ES cases, whereas coma not preceded by
convulsions was observed in 14.5% of all ES cases. The Glasgow score in
cases developing coma was consistently below 7 (median score 5), showing
that coma, as observed in the study, is an important and severe phenomenon
and not a transient decrease in consciousness level.
ES patients generally developed a deep malaise, entered a confusional status and had fever. Apathy and agitation periods were frequent and could alternate. Two thirds of the patients were tachycardic but hypotension was present in only 16% of them. Respiratory distress, consisting mainly in irregular respiratory patterns, frequently complicated the advanced phase of ES, preceding death. Nausea and vomiting were present in less than one quarter of the patients.

Signs that are usually associated with hypersensitivity reactions, such a maculopapular eruption, conjunctival hyperemia (red eye syndrome) or facial edema were observed in half, one third and one fifth of the patients, respectively.

Additional signs of CNS damage, aside from convulsions and decrease in the level of consciousness, consisted in vertigo in up to one third of the patients and psychotic manifestations (hallucinations, delirium, panic attack or aggressive behaviour), present in around one tenth of the patients. Meningeal irritation, as demonstrated by the Babinsky sign, was present in one third of the patients. Neck rigidity was however observed in only 10% of the patients. No clinical signs of coagulopathy were described.

These results show that ES follows a preferential pattern consisting of convulsions followed by coma. Variations consisting in isolated convulsions or coma without convulsions occur with lower frequencies around this main pattern. Additional signs such as fever and maculopapular cutaneous lesions were more frequent in the main subgroup developing convulsions followed by coma (52% of cases developing fever and maculopapular lesions) than in the coma (20%) and convulsive (5%) sub-groups.

This suggests that full-blown ES is an hypersensitivity-like systemic reaction consisting in multiple convulsions followed by coma and that the variations consisting in single convulsion (without or without coma) and in coma
without convulsions are less severe and incomplete expressions of the principal pattern.

The question of whether these clinical variations correspond to different etiologies or pathogenic mechanisms still needs evidence from anatomopathological studies. In their absence, the time point for occurrence and the prognosis of ES may be considered as surrogate markers for the etiology and severity of the phenomenon.

The number of doses of melarsoprol preceding the development of ES was not significantly different whether the initial manifestation of ES consisted of coma or convulsions. Furthermore, the time interval for the occurrence of ES after melarsoprol last dose before ES was similar in regard to the final outcome. In half of the patients ES was resolved within 2 days. However, when the outcome was lethal, 2/3 of the patients succumbed within one day. This suggests that there are different intensities in the pathogenic mechanisms involved. The intensity of the pathogenic mechanisms must also vary greatly in some individuals, since there are a number of patients clearly outside of the normal pattern in the time delay necessary to trigger ES and in the duration of ES. Additionally, the present study did not reproduce the higher lethality of the coma type of ES described in the literature, suggesting instead that death is related to coma, irrespective of the sequence of events leading to it. Excluding the mental type of ES, which was not observed in the study, these results give indirect evidence that the different clinical expressions of ES have a unique etiology and different severities of the pathogenic mechanisms.

Four cases were initially enrolled as belonging to the exclusively mental type of ES. They were however excluded from analysis after detecting the presence of severe mental changes previous to melarsoprol application in anamnesis. This suggests that previous descriptions of this hypothetical type of ES may suffer from an observational bias. Alternatively, we may speculate that if an exclusively mental type of ES exists, the frequency of the phenomenon is
probably much lower. Future studies applying the same stringent criteria used in the present protocol and enrolling a larger number of patients should help elucidate this issue.

11.3 Interventions for Prevention and Treatment of the Encephalopathic Syndrome

11.3.1 Preventive interventions

Interventions for the prevention of ES reflect the diversity of the drugs and schemes used for patient preparation in the different centres, which generally follow the recommendations of the National HAT control programs. The assisting clinician was however free to use the available drugs differently according to his evaluation of the patient. Unavailability of some drugs was also a problem observed in one centre (Maluku). All centres made use of a corticosteroid combined with promethazine, antimalarials and antihelmintics, although drugs in the different schemes were different. Because of the multiplicity of the drugs used and of the low number of patients enrolled in some centres, drawing firm conclusions on the role of individual drugs is not possible, but some comments may be made regarding the diverse “patient preparation” schemes.

Solid evidence that corticosteroids are efficient in ES prophylaxis remains to be obtained. The HAT control National programs in Angola and DRC have a pragmatic view of the method and support the use of prednisone for this purpose. However, the use of hydrocortisone for ES prophylaxis does not seem of advantage from the pharmacological (short half life, sodium retention action, route of administration) point of view.

The issue of the empiric use of antimalarials is complex and unclear, as demonstrated by the fact that patients receiving antimalarials in preparation may show a positive smear later during ES. The problem can not be dissociated from the issue of malaria definition and diagnosis in endemic areas. Careful and frequent monitoring of Plasmodium parasitemia could be used to increase the precision of malaria diagnosis and therapeutic
monitoring. This should be possible to implement in the treatment centres and is possibly cost-effective. The use of antimalarials should be made according to the resistance pattern prevalent in the region of origin of the patient.

Multivitamins were offered in all centres but only two offered a food supplement. In fact this food supplementation often constitutes the only source of food for the patient, especially when the patient’s family is not present in the hospital. When staying with the patient in the hospital, relatives often also have limited funds to support themselves, being outside of their village (personal observation). Better tolls are needed to access the nutritional status and to check for specific macro and micro nutrient imbalance in order to achieve optimized supplementation.

11.3.2 Therapeutic interventions
All ES patients were managed with corticosteroids except two. Half of the patients under corticosteroid therapy for ES died. The difference in ES mortality between hydrocortisone and dexamethasone was not significant. However, dexamethasone does have a more convenient pharmacological profile in terms of speed and low sodium retention actions and is the drug preferably used to treat coma associated with brain damage. This could explain the lower mortality in the group receiving this drug, since all participating centres were similiar equipped.

When present, convulsions were managed with standard doses of diazepam and/or phenobarbital. No overdosage was detected in the schemes used, which may have contributed to the low mortality associated with this drug combination, even if used simultaneously with prometazine. The difference in outcome in patients using the three diverse types of antipyretic (paracetamol, ASA or dipirone) was not significant.

Quinine, mannitol, furosemide and adrenaline were associated with a high mortality. This may hypothetically reflect the fact that patients receiving these
drugs were in a more severe condition but may also be a consequence of drug-associated iatrogenic effects.

It is difficult for the clinician to evaluate the need for aggressive quinine therapy during ES even in the presence of a positive (and most of the time low) parasitemia. Patients sometimes had already received antimalarials on admission, so that the clinical picture of malaria was potentially less severe and *Plasmodium* parasitemia lower. Fever during ES is common and thus not of much help in differential malaria diagnosis. However, few clinicians would not give quinine in a *Plasmodium* positive patient with a high fever. Quinine is a potent cardiovascular acting drug and high doses should be used with care in cardiovascular instable patients and can also contribute to promote hypoglycemia. In selected patients, quinine use seems to be ethically valid. However, further studies are needed to access to use of alternative drugs such as artesunate or combinations including this class of antimalarials, which can quickly and effectively reduce parasitemia with less toxicity.

Mannitol was used to control hypothetical brain edema. Although mannitol has an important role in managing brain edema in several conditions, its use is complicated by frequent infusions and requires careful hydroelectrolyte balance monitoring. Therefore, establishing the diagnosis of increased intracranial pressure is critical for administration of mannitol. This can be accomplished by considering clinical signs such as headache, vomiting and sudden changes in cardiac and respiratory patterns but those changes are not specific. Optic fundi examination, which was not performed in this study, is a better guide to brain edema diagnosis.

Furosemide use should be restricted to reduction of fluid overload and may be especially useful in pulmonary edema. However, pulmonary edema was not reported during ES in our population. The use of furosemide in a cardiorespiratory instable patient without careful cardiac and electrolyte monitoring can result in or aggravate hypotension, dehydratation, hipokalemia
and hyponatremia and help promote cardiac arrythmias. If used in conjunction with adrenaline, potentially fatal cardiac interactions are foreseeable, which would be difficult to diagnose and treat without electrocardiogram monitoring and biochemistry metabolic evaluation for determination of serum electrolytes. It is possible that the high mortality observed in patients receiving adrenaline, mannitol or furosemide reflects the potential drug toxicities and interactions associated with their uncontrolled usage.

Some degree of metabolic monitoring without laboratory dosages would however been possible, since a considerable number of patients had a urethral catheter and a more limited number also a nasogastric tube installed. Monitoring of fluid and caloric input/output was however not reported. Possibly, nurses were not stimulated to monitor fluid balance as this needs some know-how and also more workload. The potential benefits of maintaining a correct hydroelectrolytic and caloric input are however considerable, especially in long lasting status epilepticus and coma. Peripheral intravenous access was secured in the majority of the patients. Due advantage should be taken of this possibility in order to (at least) supply glucose, sodium, potassium, chloride and water in adequate amounts. This requires a correct estimation of the patients needs and has to take in account their frequently highly catabolic status and clinical monitoring of central venous pressure to detect fluid overload.

The potentially most useful metabolic assessment measure would have consisted in determination of arterial blood gases and pH. This requires, however, “sophisticated” equipment and is indeed useless in the absence of adequate possibilities of correcting hypoxemia, hypercapnia or acidosis. Unfortunately however, medical oxygen was never available in the centres.

Antibiotics were used when a severe bacterial infection, usually respiratory, was suspected during ES. Antibiotics used consisted mainly in ampicillin, only
once associated with gentamicine. Patients with HAT and developing ES are potentially under increased risk of developing secondary bacterial infection, especially those arising from respiratory and intra-abdominal sources. This greater susceptibility is not only associated with the immunological imbalance promoted by HAT but also with the usual critical status of ES patients. Additionally, patients have generally already received prednisone and are receiving high dose hydrocortisone or dexamethasone, which may render them even more susceptible to bacterial infection. The use of antibiotics in ES has to be carefully evaluated in respect to the epidemiology of the infecting bacterial micro-organisms and their sensitivity to antibiotics.

11.4 Risk factors for the encephalopathic syndrome

11.4.1 Clinical factors

On admission, patients reporting edema or bone pain had a significant higher risk of developing ES. Bone pain is a vague constitutional sign which is difficult to be interpreted in terms of pathogenesis. Edema (which was not confirmed as a sign during physical examination) can be related to a variety of causes. Since no evidence was found of cardiac, hepatic or renal failure in the physical examination of the patients, edema could be related to a transient neuroendocrine involvement or possibly to an also transient nutritional imbalance.

Patients in whom apathy or a depressed humour was observed also had a higher risk of ES. These manifestations could correspond to a more intense or diffuse damage to the CNS.

Patients reporting paralysis and hypoesthesia or with an observed peripheric facial paralysis or a Babinsky sign (corresponding to meningeal irritation) also showed a tendency towards more ES, but their number is insufficient for statistical significance.

The finding that normal vision, normal eye movements, and normal hearing were found to be statistically associated with ES probably reflects the low
number of patient showing abnormalities in those clinical parameters that were enrolled in the study.

The absence of association between the nutritional status, as measured by the BMI and ES or death from ES found in our study speaks against the hypothesis of malnutrition as a risk factor. The same applies to the “general status” evaluation, which includes more subjective variables than solely the nutritional status; patients in bad general status did not show a significant trend towards ES or death. Malnutrition is immunosuppressive and promotes changes in protein and micronutrient contents, which are associated with defective immune responses. The individual impact of malnutrition in HAT patients has yet to be carefully evaluated.

These findings indicate that risk factors for ES on admission are elusive and unspecific. A trend towards more evidence of neurological and possibly endocrine damage to the CNS in patients developing ES was found.

Patients developing ES had a higher risk of dying from ES if abdominal pain was reported in anamnesis. Abdominal pain is a quite unspecific symptom; in the absence of physical signs of intra-abdominal pathology or vascular problems, abdominal pain could be related to intestinal parasitosis in the context of the study. The fact that diarrhea reported in anamnesis is also associated with death from ES seems to favour this hypothesis. How this relates to death from ES is unclear but it is possible to speculate that damage to the intestinal wall, specially that associated with strongyloidiasis, amebiasis or giardiasis, which are not effectively eliminated by mebendazole or levamizole during patient preparation, may help complicate the course of ES.

Coma, respiratory distress and a Babinsky sign are associated with death from ES. Coma usually developed after convulsions and respiratory distress often appeared as a terminal event. Respiratory distress was apparently not due to iatrogenic pulmonary edema (no reference to physical signs of it were found) and should be related to metabolic acidosis and extensive CNS damage,
possibly with brain edema. Tachycardia, hypotension and to lesser extent agitation were also marginally associated with death. In addition to the severe damage to the CNS inflicted by the pathogenic process of ES itself, the complications (hypoxemia, hypercapnia, acidosis, electrolyte imbalance and cardiorespiratory failure) that are inherently associated with coma contribute (as could be expected) in establishing coma and respiratory distress as risk factors for death during ES. These findings, combined with the observed speed in reaching the final outcome of ES, calls attention for the need for quick and correct convulsion control and metabolic and cardiorespiratory evaluation followed by implementation of corrective and supportive measures in those patients from the start of ES.

Additional risk factors for death from ES consist in pruritus and conjunctivitis reported in anamnesis. The pathogenesis of pruritus in HAT is unclear but is generally accepted that it corresponds to a Peripheral Nervous system (PNS) manifestation (Cruz Ferreira and Lehman de Almeida, 1950). A CNS-related etiology for pruritus in HAT is hypothesized, especially in advanced cases (Atdouguia, personal communication). However, one must however not forget that scabies is a frequent disease in the African context. Conjunctivitis is also a common unspecific complaint, especially in the African context we are dealing with. Alternatively pruritus and conjunctivitis-like symptoms could be related to filariasis. However, filarial worms are quite easily detected in fresh or Giemsa stained blood and should not pass unnoticed by microscopists.

Edema reported in history, peripheral facial paralysis and a Babinsky sign also show a tendency for being risk factors for death from ES but the numbers are too small for statistical significance. These findings are however consistent with the fact that those symptoms and signs are also associated with an increased risk of developing ES.
These results indicate that abdominal pain, possibly associated with parasitic intestinal
(S. stercoralis, E. histolytica, G. lamblia) or blood (Filaria sp.) infection may constitute a risk factor for death during ES. Pruritus in the absence of other skin conditions could constitute evidence of a particular type of CNS or PNS damage that would render patients more susceptible to death from ES. However, for this risk factor to be confirmed in future studies patients should be carefully checked for the presence of filarial infection. Conjunctivitis reported in history could also be related to filariasis, to bacterial or viral eye infection or to vitamin deficit and is thus quite unspecific as a risk factor for death from ES. The weaker association between death from ES and evidence of a Babinsky sign possibly indicates that additional meningeal inflammation may be present in some ES patients and contribute to death.

Malaria as defined by a positive blood film was not associated with death but 70% of patients receiving quinine (thus with a clinical diagnosis of malaria) during ES died. Secondary bacterial infection from respiratory and/or intra-abdominal source was seldom reported but 62.5% of the patients receiving antibiotics during ES died. The role of concomitant infection as a risk factor for death from ES remains to be better studied and this requires minimally well equipped laboratory facilities, including access to microbiological techniques.

11.4.2 Laboratory data

Laboratory evaluation on admission showed that none of the measured biological markers is clearly associated with the triggering or pathogenesis of ES. A trend towards an association between trypanosomes in lymph and ES and trypanosomes in CSF and death from ES was observed but the size of the sample is too small for statistical significance. CSF WBC count on admission was analysed dividing the results of the count in quartiles. The limit for the first quartile was found to be 100 WBC cells in CSF. Additionally, the more conventional (and arbitrary) division of WBC count in CSF (less than 20 cells, 21 to 100 cells and more than 100 cells in CSF) was also used in
analysis (data not shown). The WBC count in CSF was not found to constitute a risk for ES or death. This indicates that WBC count in CSF is not a reliable marker of the peculiar CNS damage in HAT that predisposes to ES and that better laboratory indicators are needed. Possibly, markers of host immune response such as galactocerebrosides, antimyelin and antineurofibrillary antibodies, or cytokines dosage in CSF (and blood) have a greater chance of being associated with ES, but these tests are not presently easily available.

The laboratory evaluation of ES cases, performed in 1/5 of the patients, did not show severe anemia or significant changes in leukocyte blood count. Unfortunately, neither the leukocyte differential blood count nor the platelet counts were available. No increase above the baseline count of WBC count in CSF was observed during ES. Half of the patients, however, had glucose levels in CSF close or below to the accepted inferior limit of 50 mg/dL. Simultaneous dosage of glucose in blood was not available but the values observed in CSF could indicate that hypoglicorraquia (and possibly hypoglycemia) and is a frequent complication of ES. A CSF Gram stain obtained in 11 cases was negative, eliminating (at least with some degree of confidence) meningoencephalitis of bacterial origin as a confounder in our ES population.

Additional laboratory parameters for hepatic, renal, respiratory and metabolic evaluation would have allowed a more precise guidance of ES therapy but they were not available in the Centres. A simple parasitological stool analysis would be of much use to guide effective antiparasitary therapy on admission.
II.5 HLA type

Haplotype C*14 / B*15 was associated with a risk more than six and a half times higher of developing ES. The association is significant (p value 0.008) but the upper limit of the CI is considerably large.

Haplotype A*23 / C*14, was also found to be potentially associated with ES (p value less than 0.05), with a risk nearly 10 times greater of developing ES with this haplotype but the inferior limit of the CI is below one, suggesting that a greater number of patients is needed for confirmation.

A*23 / B*15 and DR*07 / B*58, show a tendency for association with ES but the level of significance for these allele combinations is above a p value of 0.05. Furthermore, the <1 inferior limit of the CI for those haplotypes also indicates that size sample has to be increased to confirm these associations.

One possible reason for the diversity of the potential haplotypes detected could be related to the ethnic heterogeneity of the study population. In Angola, patients belonged to the Kimbundo, Kikongo, Ovimbundo, and Bakongo ethnic groups. The determination of ethnic groups was not available in the DRC, since only the village of birth of the patient was recorded, due to difficulties found in attributing the correct ethnic group to individuals. Ethnic matching could possibly bring more precise results but was not possible in view of the multicentre characteristic of the study and the limited time frame. A much larger time frame would be needed to enrol a sufficient number of ES patients from each ethnic group to allow statistical comparisons. The other possibility is that the genetic component of ES is polygenic or associated with a single major locus with other loci of small effect and not oligogenic.

Although the level of association between haplotype C*14 / B*15 and ES is significant, replication of these results is needed to confirm the alleles and haplotypes involved in ES, as usually recommended in association studies.
12 CONCLUSIONS

A case control study with prospective acquisition of clinical data comparing late stage *T. gambiense* sleeping sickness patients treated with melarsoprol and developing or not developing an encephalopathic syndrome was conducted in 6 HAT treatment centres, 3 in Angola and 3 in the DRC, between June 2002 and November 2003. 76 out of the programmed 100 ES cases were enrolled. According to the stringent definition criteria, 69 cases with 207 controls were accepted for analysis.

Study methodology was found to perform adequately. The implemented Case Report Form, although complex and requiring the collaboration of a full-time clinician, allowed the collection of detailed clinical data on admission and during ES and may be used as a tool in future studies on HAT and ES.

The capillary blood sample collection method using a special filter paper (Generation® Sample Collection Cards) proved to be easy and adequate to the field conditions. DNA of sufficient quality and quantity could be extracted from the Generation® cards more than 9 months after blood collection, without the need for blood component separation in the field or liquid nitrogen for sample preservation.

Class I HLA-A, HLA-B and HLA-Cw molecules and Class II molecules of the HLA-DRB1 category were typed using two different commercially available PCR-based reverse line blot assays that detect specific target DNA sequences by means of multiple immobilized sequence-specific oligonucleotide probes. Alleles could be correctly determined by these methods from a total number of 62 ES cases and 189 controls. The remaining samples showed typing ambiguities. Clarification of detected ambiguities by high definition typing or gene sequencing would have optimized statistical power but was outside of the possibilities of the present study.

ES followed preferentially a clinical pattern consisting of multiple convulsions followed in half of the cases by a deep coma. Patients with the main pattern
of convulsions followed by coma had a higher incidence of fever and maculopapular eruptions and a higher mortality from ES. The clinical picture accompanying convulsions and the subsequent development of coma consisted mainly in a profound malaise, a confusional status with apathy and agitation sometimes alternating and fever. Tachycardia was common but hypotension developed in only a reduced number of patients. A maculopapular cutaneous eruption, conjunctival hyperemia or facial edema, usually associated with hypersensitivity reactions, were observed in an important percentage of patients. Respiratory distress, consisting mainly in acidotic and irregular respiratory patterns frequently often preceded death. Meningeal irritation during ES, as demonstrated by the Babinsky sign, was present in one third of the patients. No clinical signs of coagulopathy were described. Episodes consisting of isolated convulsions without coma and of coma not preceded by convulsions were also described in a smaller number of patients. This data suggests that full-blown ES consists in convulsions followed by coma and that the other manifestations are incomplete expressions of the main pattern.

Nearly half of the patients developed ES after melarsoprol 10th dose. ES diagnosis was established within 48 hours after the last melarsoprol triggering dose in ¼ of the patients. The final outcome of ES was usually defined within two days, and 80% of patients died within 3 days. Death was the final outcome of ES in nearly half of the patients. Sequelae of ES were detected in less than 1/3 of the patients and were generally not severe.

Since anatomopathological studies were not performed, the time point for occurrence of ES and the prognosis were used as surrogate markers for the etiology and pathological severity of the phenomenon. The number of doses for initiation of coma or convulsions did not significantly differ. The time interval for development of ES after the last melarsoprol triggering dose was similar in patients surviving or dying from ES. The final outcome tended to be defined in a shorter period of time in patients dying from ES. A number of
patients were outside of the normal pattern in the time delay necessary to
trigger ES and in the duration of ES. Excluding the mental type of ES, which
was not observed in the study, these results give indirect evidence that the
different clinical expressions of ES probably have a common immune
etiology. The different clinical pictures appear to correspond to different
severities of the pathogenic mechanisms. We may speculate that if the mental
form of ES indeed exists and is not merely the consequence of an
observational bias introduced in previous reports, its frequency is possibly
much lower than the main pattern of ES we observed.

Laboratory evaluation of ES, which is uncommonly found in the literature,
showed that hemoglobin and leukocyte count in blood are not
classically changed in 15 ES patients. A lumbar puncture CSF was
performed within the duration of ES in 15 patients. No increase above the
baseline count of WBC count in CSF was observed during ES, although this
parameter is difficult to evaluate after administration of melarsoprol.
However, glucose levels in CSF close or below to the accepted inferior limit
of 50 mg/dL, observed in half of the patients, seems to indicate that
hypoglicorraquia may be a frequent complication of ES. The CSF Gram stain
obtained in 14 ES patients was always negative, showing with some degree of
confidence that ES patients in the study did not suffer from a confounding
bacterial meningoencephalitis.

The analysis of risk factors for ES and death from ES on admission showed
that edema, bone pain, apathy and a depressed humour were associated with
the development of ES. Hypoesthesia, a peripheric facial paralysis and a
Babinsky sign showed a tendency towards association with ES.

Abdominal pain, diarrhea, pruritus and conjunctivitis on admission and coma,
respiratory distress and a Babinsky sign during ES were associated with
mortality. Edema in history, peripheric facial paralysis and a Babinsky sign on
admission showed a trend towards association with death from ES.
Evidence for association between the remaining studied demographic, clinical and laboratory variables, including the presence of trypanosomes in the diverse body compartments, the number of WBC in CSF and microbiologically confirmed parasitic (including malaria) or suspected bacterial infection was missing.

These findings give evidence that patients showing certain symptoms or signs, which may be interpreted as corresponding to a particular type of CNS damage, tend to develop ES or dye from it. Some of this symptoms and signs are elusive and others need a correct neurological evaluation of the patient. Additionally, abdominal pain, possibly reflecting intestinal parasitic infection and pruritus, possibly reflecting filarial blood infection, could be associated with mortality from ES. These hypothesis needs however laboratory confirmation of the potentially involved pathogens, including S. stercoralis, E. histolytica, G. lambia and Filaria sp. Better indicators of the host-parasite interaction and especially of the immune response to trypanosomal infection, particularly in the CNS, are needed for a more precise determination of ES susceptibility.

Haplotype C*14/B*15 was significantly associated with ES. Three additional haplotypes (A*23/B*15, A*23/C*14 and DR*07/B*58) showed lesser degrees of association with ES. This finding, although needing confirmation in additional studies and other geographical settings, shows that a genetic marker could be available for ES and point the way for future research on the etiology and pathogenesis of ES.

The correct assessment of the role of preventive or therapeutic interventions in ES needs controlled trials. Since this was a non-interventional study and given the diversity of drugs used for prevention and treatment of ES, only a limited analysis of their role was possible.

Patients usually received prednisone, antimalarials, antihelmintics and multivitamins in prophylaxis. Determination of the role of antimalarials and
anthelminthics in ES needs careful microbiological assessment on admission. The empiric interventions used are not efficient against certain pathogens that could be involved in ES pathogenesis.

Generally patients received corticosteroids for therapy of ES and half of them died. Dexamethasone offers a more adequate pharmacological profile and this could explain the tendency for a lower mortality observed with this drug, when compared to hydrocortisone, which was the most frequently used corticoid. Quinine, furosemide, mannitol, adrenaline and, to a lesser extent, antibiotic usage during ES was associated with a high mortality. The multiple potential adverse effects and interactions observed with these drugs makes their use hazardous in a critically ill ES patient. Prescription of these drugs should probably be limited to patients showing solid evidence of malaria, fluid overload, raised intracranial pressure, shock or severe bacterial infection.

Some degree of clinical monitoring and correction of fluid and caloric balance would have been possible since intravenous fluids, a urethral catheter, and a nasogastric tube were generally available. Given the fast development of ES, quickness and precision in the diagnosis and treatment of complications developing during convulsions and coma and the avoidance of unnecessary drugs and drug combinations seem critical in ES patients and may improve the prognosis of ES. Optimizing clinical skills for diagnosis of complications should include the determination of the fluid balance and an optic fundi examination. Laboratorial monitoring of metabolic indicators, including electrolytic balance, arterial blood gases and pH determination and microbial pathogens would have been useful but was not available. Laboratory facilities found in the centres were technically very limited but could, with a small additional effort, be at least able to perform a correct parasitological diagnosis. Medical oxygen was however the most important missing resource for treatment of convulsing or comatose ES patients.
PART IV
GENERAL DISCUSSION, CONCLUSIONS AND PERSPECTIVES

13 THE ENCEPHALOPATHIC SYNDROME: A CLINICAL ENIGMA

A dual approach was used to tackle the enigma of the melarsoprol-related encephalopathic syndrome in human African Trypanosomiasis.

The first consisted in performing a systematic review of published and unpublished literature on ES, using the Cochrane Group methodology for systematic reviews. Around 2500 references were primarily scanned for relevance. Among the 157 references considered potentially relevant, 46 datasets met the inclusion criteria and were accepted for analysis. The accepted datasets described a total number of 562 ES patients.

The second consisted in a case control, non-interventive, multicentre clinical study on ES, in late stage Gambiense HAT patients. Unlike in the majority of available studies on ES, patient clinical data was prospectively acquired. The existence of an association between ES and the HLA complex was explored. Class I HLA-A, HLA-B and Cw and Class II HLA-DR alleles were determined using state-of the-art DNA detection with PCR sequence-specific oligonucleotide probes. The study was conducted in 6 HAT treatment centres in Angola and in the DRC, between June 2002 and November 2003. A final number of 69 ES cases and 207 controls were included for analysis of clinical variables and HLA typing.

13.1 DEFINITION OF ES

Three hypothetically different forms of ES consisting of convulsions, coma or exclusively mental severe psychotic manifestations, respectively, are reported in the literature. The independent existence of these three types of ES has been postulated based on a very limited number of anatomopathological studies. Should such a distinction be correct, they might represent different independent phenomena.
The hypothetical form of ES consisting exclusively in mental changes was not observed in the clinical study. References in the literature to this hypothetical form of ES may constitute an observational bias, with mental changes already present but not detected in the patient history or under-evaluated in clinical examination. Alternatively, ES with exclusively mental manifestations could have a much lower frequency than the main preferential pattern.

Evidence obtained from both the systematic review and the clinical study indicates that ES follows preferentially a main clinical pattern consisting in convulsions followed by coma. Fever and a cutaneous maculopapular rash were more frequently observed in the clinical study in patients with these manifestations. We consider this to be the main clinical pattern of ES, as it covers the full spectrum of the possible manifestations of the phenomenon.

Evidence of the existence of ES episodes characterized only by convulsions without coma or by coma without convulsions was also obtained from the systematic review and from the clinical study. The frequency of these incomplete manifestations of ES is lower than the one associated with full-blown ES.

The few available anatomopathological studies in patients with the coma or convulsive manifestations of ES show that the phenomenon results in diverse degrees of severity of a hemorrhagic leukencephalitis, with macro and micro hemorrhages around small vessels of the brain, which show fibrinoid necrosis. The changes may be observed in several regions of the CNS, including various anatomic structures in the mid-brain and brain stem. Brain edema and hypoxic brain damage are observed also in several degrees of severity. Evidence of a type of cerebellar histological damage suggestive of hypoglycemia was found in one of the very few available anatomopathological studies on ES. It is worth mentioning that the last clinical pathological correlation study was performed in 1986, in the Ivory Coast.
13.2 CHARACTERISTICS OF THE ENCEPHALOPATHIC SYNDROME

The combined findings of the systematic review and the clinical study show that a profound malaise, mental confusion, apathy, agitation and tachycardia are common features of ES. Respiratory distress with irregular respiratory patterns often follows convulsions or coma and precedes death. Hypotension or cardiovascular shock and signs of meningeal irritation may be also present. No clinical signs of coagulopathy were described in the clinical study but were found in the literature. Additional signs consisting in conjunctival hyperemia and facial edema were present in up to 1/3 of the patients in the clinical study, suggesting a hypersensitivity reaction.

ES is referred in the literature as appearing “like a bolt in the blue”. However, the systematic review indicates that the development or intensification of fever, headache, nausea, vomiting, dizziness, tremors and possibly conjunctival hyperemia may herald ES. Although heralding signs of ES were requested in the clinical study, except for fever, they were generally not reported. This may reflect the fact that heralding signs are transient. The clinical study could however establish that in 3/4 of the patients, ES occurs within less than 48 hours after the last melarsoprol dose, and can occur as rapidly as 10 to 15 minutes after the last melarsoprol dose. These facts stress the need for careful patient surveillance while under treatment with melarsoprol.

The final outcome of ES is in average determined within 2 days. Patients dying from ES in the clinical study tended to reach the final outcome more quickly than those surviving, and within 3 days 80% of the patients were dead. This again calls attention for the need of adequate patient surveillance so that a prompt diagnosis of ES can be made and adequate measures are taken. Surviving patients usually recovered completely from ES. Sequelae were observed in 1/3 of the patients in the clinical study and were generally not severe, which is in accordance with data in the systematic review. The
resumption of melarsoprol after an ES episode is reported in the literature in 43 patients and did not result in additional complications.

A lethal outcome was the result of ES in nearly half of the cases in the clinical study, with non-significant differences among the participating centres. This is within the range of mortality for ES found in the literature in Gambiense HAT, although huge variations across countries and regions were also observed. The lack of adequate criteria for ES diagnosis, the erroneous attribution of the cause of death, and the quality of care for critical patients can act as confounders in the estimation of ES incidence and mortality. This may explain the geographical variations observed in ES incidence and mortality observed in the systematic review.

Despite considerable efforts, the cause for the seasonal fluctuation in ES incidence and mortality, which has been described exclusively in the Northern Uganda region, remains to be identified. The main hypothesis for this peculiar phenomenon includes exogenous factors that would increase the patient susceptibility towards ES. Seasonally fluctuating nutritional factors, including clinically difficult to detect micronutrient imbalance (especially selenium) and undiagnosed concomitant infection with an additional viral or bacterial pathogen are working hypothesis that need to be explored.

13.3 Etiology and pathogenesis
Since anatomopathological studies on ES are so few, evidence for the etiology and the pathogenesis must be acquired indirectly. Furthermore, no biological marker of ES is available. Clinical studies establishing the time-point for the occurrence of ES and its prognosis, which may be considered surrogate markers for ES etiology and pathogenesis, were used to gain insight into those aspects.

ES occurs most often 10 days after the initial melarsoprol administration, and this appears to be independent of the dose of melarsoprol received by the patient. This was demonstrated with the introduction of a 10 consecutive day
concise schedule for melarsoprol administration (IMPAMEL). However, ES may occur at any time-point after the first melarsoprol administration and up to 30 days after the last one. Patients in the clinical study were all treated with this new concise melarsoprol scheme. They showed a similar time point for the triggering of ES and a similar prognosis for episodes starting with coma or convulsions. These data suggest that the diverse modes of presentation of ES have a unique etiology and that different intensities in the pathogenic process may exist, leading to differently severe clinical features.

Similarities between melarsoprol-related ES and other similar conditions were found in the systematic review. Encephalopathic-like syndromes are described in association with oncochercosis and loiasis, and with various arsenical and non-arsenical drugs. In HAT, ES-like conditions are described with practically any other antitrypanosome drug and in patients in both stages of disease. The number of these observations is however very small and no pathological studies are available. Two conditions bearing similarities with ES and with correct pathological documentation available were found in the literature. Arsenical encephalopathy and acute hemorrhagic leukoencephalitis (AHLE, or Hurst disease) were found to have CNS histopathological features consistently similar to the ones described in melarsoprol-related ES in HAT. Arsenical encephalopathy was associated with the use of arsenicals mainly during the treatment of syphilis with these compounds. No firm etiology of the phenomenon could ever be established, but an immune reaction related to individually determined drug toxicity was postulated. Clinical features common to the three conditions include the unpredictability of the phenomenon, the suddenness in its onset, the variability of the manifestations, the lack of biological markers and the bad prognosis. The main difference between ES, arsenical encephalopathy and AHLE is the much higher frequency in which melarsoprol-related ES is observed. As described in the discussion of the etiology of ES in Chapter 6, AHLE is a very rare CNS condition triggered by viral or bacterial challenge. AHLE is thought to constitute the extreme and lethal end of the spectrum of acute
disseminated encephalomyelitis (ADEM), an uncommon demyelinating
disease of the CNS. ADEM is considered by some authors as part of the
spectrum of multiple sclerosis. Evidence from human studies and animal
models suggest that AHLE is an immune reaction mediated by T-cells,
possibly in synergy with antibodies. The accepted theory is that an
autoaggressive attack on CNS structures is launched, possibly misdirected by
molecular mimicry with microbial epitopes. Alternatively, a non-specific
activation of an autoreactive T-cell clone is postulated.

Additional data from animal models that reproduce to a certain extent the
findings of ES in man were found in the systematic review. Valuable evidence
from those studies was also considered. In the murine animal model that
more closely reproduces ES, astrocytes were found to be involved in the
establishment of brain damage and the phenomenon can be partially inhibited
by the anti-inflammatory and immunosuppressive drug azathioprine.

These combined findings favour the hypothesis that the etiology of
melarsoprol-related ES is a T-cell mediated immune response occurring
within the CNS, in which the endothelium of brain vessels and microglial cells
in brain parenchyma are mistargeted for immune attack. This results in loss of
endothelial integrity, perivascular effusions, edema and immune cell
proliferation and infiltration. CNS necrosis and demyelination are the
consequence of the intensive damage inflicted to the CNS observed in the
severe forms of ES.

The pathogenesis we propose for ES, based on data found in the literature
review, is complex. Once ES is triggered, activation and recruitment of
immune cells in the CNS starts in certain susceptible areas of the CNS,
possibly involving circulating T and B cells, as well as resident immune cells in
the brain, mainly astrocytes. Immune mediators such cytokines, prostanoid
products and nitric oxide are released, resulting in uncontrolled amplification
of the endothelial and brain parenchyma cell damage. The phenomenon
occurs with diverse intensity in different parts of the brain, depending on factors that may be host or parasite-related, corresponding to the different clinical presentations. Brain hypoxia, edema and hypovolemia result in convulsions and/or coma. Subsequently, metabolic consequences of the convulsions and coma, including acidosis, hypoglycemia and cardiorespiratory instability further contribute to hypoxemia and brain damage. Additional complications are also prone to develop in such critically ill patients, such as bacterial or parasitic infection. Furthermore, the drugs needed to manage increased intracranial pressure, fluid overload and hypotension and infection have many potential severe adverse effects. Deficiently monitored or uncontrolled usage of these drugs may also contribute to the frequently fatal outcome of ES.

The factors that may confer a given individual susceptibility towards ES are multiple and probably in close relation with the complex host-parasite-melarsoprol interaction present during the treatment of HAT, particularly in late stage disease.

Human trypanosomes deeply affect the host immunological balance. Changes in TNF-α, INF-γ, IL-1β, IL-6, IL-10, PGE, and NO production are observed in blood and in the CNS of late stage HAT patients. Important documented effects of the combined systemic and CNS cytokine imbalance include altered blood brain barrier (BBB) permeability and the upregulation of existing autoreactive cell clones. Furthermore, antibodies directed to myelin, galactocerebrosides and neurofilaments have been demonstrated in late stage HAT. Evidence has also been obtained showing that CSF from late stage HAT patients promotes apoptosis in microglia and endothelial cells and contain potent inducers of the cell death receptor Fas (CD 95). This could be related to the recently identified trypanosomal apoptotic factor (TAF) which is thought to mediate human brain vascular endothelium apoptosis. These findings indicate that trypanosomal infection establishes a favourable environment within the CNS for autoimmune phenomena to occur. The
existence of peculiar strains of trypanosomes capable of inducing a more intense immunological imbalance, or with an increased ability of persistence in certain structures of the brain, namely those where the BBB is weaker such as the choroid plexus, can be hypothesized. These trypanosome strains would prepare a more favourable CNS environment for the development of ES.

Individual susceptibility towards ES could be related to differences in melarsoprol metabolism. Correctly performing tools for determination of melarsoprol pharmacokinetics were made available only recently. A great variability in active melarsoprol metabolites levels in the CSF has been demonstrated. This is thought to be correlated to the degree of damage to the BBB. Greater permeability of the BBB could result in enhanced penetration and higher levels of melarsoprol or melarsoprol metabolites in the CNS. Studies restricted to the animal model show that melarsoprol biotransformation is mainly made by glutathione (GSH) conjugation and glucuronidation and that GHS may be depleted from tissues during melarsoprol biotransformation. In humans, melarsen is the main metabolite of melarsoprol and was found to be covalently bound to serum proteins. Limited pharmacological data on melarsoprol and melarsoprol metabolites in blood and CSF of ES patients indicate that a prolongation in the half-life of melarsoprol may be present in these patients. A major difficulty in obtaining detailed pharmacological data on melarsoprol pharmacokinetics in CSF is the need for additional lumbar punctures in already critically ill patients. Comparative melarsoprol pharmacokinetic studies in patients suffering or not from ES are however needed to establish the existence of patients with anomalies in melarsoprol levels, especially in the CNS. In the clinical study CSF was obtained within the duration of ES in a considerable number of patients, demonstrating that these studies are feasible if a reasonably good standard of patient management exists.

Future studies on melarsoprol metabolism and ES should also take into account the existence of genetically determined isoforms of cytochrome
P450, which can significantly change the characteristics of individual drug requirements. Furthermore, cytochrome P450 isoforms are also expressed in certain areas of the brain and can modify the local action or concentration of CNS acting drugs. These areas could correspond to the areas where ES is triggered.

An individual genetic predisposition to develop the type of immune response against trypanosomal challenge that creates a favourable environment in the CNS and leads to ES appears as a valid working hypothesis. In humans, the HLA complex is a major determinant of the immune response.

In 62 ES cases enrolled in the clinical study in whom HLA Class I A, B, and Cw and class II DR alleles could be correctly typed, haplotype C*14 / B*15 was expressed significantly more frequently than in controls. The expression of this haplotype is associated with a risk more than 6.5 times greater of developing ES. Haplotypes A*23 / C*14, A*23 / B*15 and DR*07 / B*58 also showed a trend for association with ES, but sample size was insufficient for adequate statistical power. Alternatively, we may consider that the genetic component of ES could be polygenic or associated with a single major locus with other loci of small effect. As usual in genetic association studies, confirmation of these results is needed in other populations. The ethnical diversity observed in the clinical study was considerable and the low numbers in the different ethnic groups was not sufficient to allow for sufficiently statistically powered ethnic stratification. Since the physical map of the HLA complex genes is now available it is possible to explore the molecular basis of ES. Linkage of the Class II allele found in the study with Class III genes or non-HLA genes involved in the immune response, especially those related to the synthesis of the cytokines and mediators described in late stage HAT, should be explored to gain insight into the etiology and pathogenesis of ES.
14 THE SEARCH FOR RISK FACTORS FOR THE ENCEPHALOPATHIC SYNDROME

Clinicians need valid and consistent evidence on the risk factors that may indicate a patient’s greater susceptibility to develop or die from ES. Preventive and therapeutic interventions can only be effectively designed if the risk factors associated with ES are determined. Even if eflornithine is presently more easily available, few HAT treatment centres are or will be in the near future logistically able to treat every late stage patient with this drug. The decision of withholding melarsoprol and individually treating a patient with eflornithine can only be rationally taken based on the correct identification of ES-susceptible patients.

The systematic review showed that valid and robust evidence of the risk factors for ES or death from ES is limited. Data on ES came mainly from case series and case reports, and was often circumstantial. Controlled studies are few and show conflicting evidence. Furthermore, the application of different statistical methods and of frequently underpowered stratification analysis does not allow drawing solid evidence from the studies. The complexity of the variables involved in ES and the high number of drugs used in preventive and therapeutic interventions introduce many confounders. Valid evidence was found that gender, the ethnic group and the treatment schedule do not influence the risk for ES or the case fatality rate. Concomitant infection appeared consistently associated with an increased risk of ES and death. BAL administration was found to be deleterious in ES treatment. Patient age, the form of HAT (Rhodesiense or Gambiense), the type of presumed manifestation of ES (coma, convulsions or mental) and alcohol consumption appeared as being possibly associated with ES and/or CFR. The validity of evidence for the role of the patient general status on admission, of the laboratory markers of HAT (trypanosomes in blood, lymph and CSF and WBC count in CSF) and of the interventions for prevention and treatment of ES (including corticosteroids) was found to be conflicting and unreliable. In particular, a logistic regression with pooled data from 29
datasets for preventive interventions and from 25 datasets for therapeutic interventions showed that no single intervention or combination of interventions had a protective effect in the prevention or outcome of ES. This finding, although potentially resulting from a defective statistical model related to the presence of many confounders, offers a challenging view and indicates the need for the correct evaluation of preventive and therapeutic interventions in ES.

On the other hand, the prospective and detailed clinical data collection in the clinical study allowed a methodological correct analysis of the clinical risk factors for ES and death from ES.

The results obtained in the clinical study indicate that clinical manifestations which may correspond to a particular type of damage to the CNS, consisting in transient edema (possibly of neuroendocrine origin), changes in mood and in mental humour and pain are associated with the development of ES. Hypoesthesia, paralysis, signs of meningeal irritation and pruritus are also possibly correlated to a certain type of CNS damage and a greater risk of ES.

The finding of that abdominal pain and a previous history of diarrhea is associated with a significantly higher risk of dying from ES could be related to an intestinal parasitosis present in those patients. Since all patients received mebendazole or levamizole on admission and none of them had a parasitological stool analysis performed, we may speculate that infection with pathogens that are not effectively treated by these compounds, such as S. stercoralis, E. bistolytica, G. lamblia or Schistosoma sp. could be risk factors for death from ES.

Transient edema, pruritus and conjunctivitis in history also tended to be associated with death from ES. This again suggests that some particular type of CNS damage may be present in ES-prone patients. The correct evaluation of pruritus and conjunctivitis as risk factors should however include a differential diagnosis with filarial infection, scabies and specific eye infections.
Coma, respiratory distress and to a lesser extent tachycardia and hypotension were associated with death from ES, reflecting the intrinsically high mortality related to the severe CNS damage present in ES.

The role of well documented concomitant infection in ES risk and mortality is difficult to establish in the clinical study, partially due to the prophylactic use of antimalarials. However, 70% of the patients with a clinical diagnosis of malaria (receiving quinine) and slightly more than 60% of the patients treated with an antibiotic during ES died. The role of concomitant infections during ES is inherently associated with their diagnosis. The limited laboratory facilities present in most of the HAT treatment centres severely jeopardize the acquisition of good quality data on the role of concomitant infection. All HAT centres are however able to perform a blood smear and in some of them a parasitological stool analysis could probably be performed with limited additional training. A CSF Gram stain was obtained in 20% of the ES patients in the clinical study. Measures aimed at optimizing the laboratory resources in selected centres, consisting for instance in serial *Plasmodium* thick smears for malaria diagnosis, a systematic screening for intestinal parasites and CSF Gram stain in ES patients could allow a more correct evaluation of the role of concomitant infection as a risk factor for ES and death from ES.

All other demographic and clinical variables in the study, including the general and nutritional status (indicated by the BMI) on admission, the presence of trypanosomes in body compartments and the number of WBC in CSF were not associated with ES or death from ES.

The combined results of the systematic review and the clinical study indicate that risk factors for ES and death from ES are elusive. To explore the statistical trends found in the systematic review and in the clinical study with increased power and precision, future studies should include a sufficiently large enough number of ES patients and controls for adequate statistical power in selected subgroups.
The association between HLA molecules and patients expressing the phenotype of ES found in our study indicates that susceptibility towards ES may be of genetic origin and highly individual. The determination of alleles requires DNA extraction, which is impossible to perform in the field with presently existing methods. Thus, although original, and potentially useful in exploring the molecular basis for the etiology and pathogenesis of ES, this finding has presently no clinical application. Clinicians will still have to rely in their clinical skills to identify the clinical signs that are associated with susceptibility to develop or die from ES. Based in the findings of our clinical study the improvement of neurological observation skills appears to be especially relevant.

15 IMPROVEMENT OF THE DIAGNOSIS, PREVENTION AND MANAGEMENT OF THE ENCEPHALOPATHIC SYNDROME

15.1 Diagnosis
Our findings indicate that a diagnosis of ES should be established in a HAT patient undergoing or having finished melarsoprol therapy in the previous 30 days and developing de novo convulsions and/or coma. In patients with convulsions, severe mental changes or a decreased level of consciousness before the administration of melarsoprol and developing convulsions or coma during or after drug application, ES diagnosis is not entirely clear. Those patients require careful evaluation and follow-up.

The question of whether exclusive mental changes should be included in the spectrum of ES or constitute an intensification of the CNS manifestations of HAT needs clarification by means of adequately sized studies with stringent inclusion and exclusion criteria to detect the presence of these mental changes before melarsoprol administration. Corticosteroid use in this type of severe mental manifestations appears like highly questionable as it may aggravate the psychotic manifestations.
Establishing the existence of increased intracranial pressure (ICP) in an ES patient is critical for correct management. Increased ICP can be correctly identified by the presence of papilledema in optic fundi examination. Computerized tomographic (CT) scans of the CNS are most helpful in establishing the diagnosis of increased ICP, but they are rarely available in the context where ES patients are managed. Thus the clinician has again to rely basically on his clinical skills to diagnose raised ICP during ES.

The analysis of blood and CSF parameters obtained during ES in patients enrolled in the clinical study confirmed that no diagnostic test is presently available to characterise ES. It is therefore important to correctly diagnose conditions that potentially confound the diagnosis of ES. The differential diagnosis includes several viral, bacterial, fungal and parasitic CNS diseases prevalent in tropical Africa that can take advantage of the immunedisregulation present in late stage HAT, which is potentially aggravated by corticoid administration during preparation and for ES management. Performing a lumbar puncture during ES may be a dangerous procedure and should be only undertaken by skilled hands, once increased ICP has been ruled out or controlled. Microbiological methods for correct pathogen identification in CSF are unfortunately seldom available in HAT treatment centres, but a CSF Gram stain is generally possible to obtain and rules out, at least with some degree of confidence, the existence of potentially treatable bacterial meningitis. A differential WBC count and platelet estimation in blood is also potentially useful in the patient’s differential diagnosis and follow-up. These parameters can be obtained with adequate additional training of the experienced microscopists usually existing in HAT treatment facilities.

The precise evaluation of the many and severe metabolic complications of status epilepticus and coma requires adequate laboratory facilities for determination of arterial blood gases, pH, serum electrolytes and biochemical parameters. Low glucose levels in CSF and and hypoglycemia were identified
as potentially frequent complications of ES. A simple strip test for glucose would be of advantage in the diagnosis of this complication. Hypoxemia was found to be the most important metabolic complication of ES. Laboratory facilities in the African context are generally very poorly equipped and do not allow this type of laboratory evaluation. Clinicians must thus once again heavily rely on clinical skills to correctly evaluate the patient cardiorespiratory status and fluid balance and to be able to effectively correct disturbances. Correcting hypoxemia requires however medical oxygen, which unfortunately was found to be rarely available in HAT treatment centres in the past and possibly even more so nowadays.

In the African context, the perspective of using Magnetic Resonance Imaging, which has been shown to be potentially useful in differentiating between ES and HAT encephalitis is very limited. Polysomnographic recordings are presently possible to obtain and interpret in the field, thanks to available portable electroencephalogram, electromyogram and electro-oculogram methodology. Polysomnographic recordings allow the identification of sleep/wake changes that correlate well to the intensity of CNS damage in HAT and are potentially useful in monitoring the therapeutic response and in identifying patients at risk of developing ES. Obviously, better laboratory tools are also needed to correctly identify ES susceptible patients, to diagnose the condition and to monitor therapy. Recently developed markers of the severity of CNS damage and autoimmune activity are available in the research setting. Although these markers could theoretically correlate better with ES susceptibility, the techniques are presently difficult to apply in the field and need validation.

Even if better diagnostic markers of ES become available in the future, obtaining a correct clinical diagnosis of ES based on a careful evaluation of the patient will remain crucial for the management of the complication and to avoid unnecessary and potentially dangerous interventions. Mis-evaluating convulsions or severe mental changes in the patient history may confound the
diagnosis of ES and may incite the clinician to launch inadequate treatment of ES. Careful patient observation may also improve the patient’s prognosis once ES is triggered.

15.2 Prevention and Management

The role of the currently recommended preventive and therapeutic interventions in ES, all of them empirically developed, urgently needs correct assessment. Evidence of the efficacy and safety of the many different drug schemes used in patient preparation and treatment of ES would lead to their more rational use. The correct assessment of the role of the different drugs used in ES prophylaxis and treatment needs correctly designed and powered clinical trials.

Antiparasitic drugs, including blood acting antitrypanocidal drugs, antimalarials and anthelminthics are generally accepted as useful in patient preparation before melarsoprol is given. Pentamidine and suramin were considered to reduce melarsoprol-associated fever especially during the first applications of the arsenical but their role in ES prevention is undetermined. The use of pentamidine or suramin before melarsoprol in Gambiense disease is now largely abandoned. The role of antimalarials is questionable as shown by the finding of a positive Plasmodium smear after their empirical use on patient admission. The question is related to the problem of the correct diagnosis of malaria in an endemic context and to the prevalent Plasmodium resistance pattern in a given area. Chloroquine, on the other hand, has potentially deleterious effects since this drug has been shown to increase pro-inflammatory cytokine production in the CNS. Antihelmintic drugs used in patient preparation presently consist mainly in mebendazole, although other drugs like levamizole may be used. Potentially important intestinal protozoan like G. lamblia and E. histolytica, and helminths such as S. stercoralis and Schistosoma sp. are not efficiently treated by these drugs. The validity and cost-effectiveness of mebendazole and other drugs aimed at reducing additional concomitant intestinal and blood parasitic (including Filaria sp.) load in HAT
patients should be correctly assessed, with accurate blood and stool parasitological analysis.

Corticosteroids are capable of modifying the immune response and are used in a variety of auto-immune and inflammatory conditions, including brain edema. Corticosteroids are presently accepted as having a beneficial role in ES prophylaxis and treatment. In the clinical study, all clinicians used a corticosteroid for prophylaxis of ES and in all except two patients a corticoid was also used to manage ES. However, corticosteroid use, especially in ES prophylaxis, needs correct assessment, since data on the effectiveness of this type of drug is conflicting. Corticosteroid use for treatment of ES is less controversial, although the type of corticoid, the dosage and the duration of therapy remains to be correctly established. Dexamethasone, which was less used than hydrocortisone in the clinical study, offers a better pharmacologic profile and is the most widely drug used in similar CNS conditions. Very high doses of dexamethasone or methylprednisolone have been successfully used in the management of ADEM and AHLE. In these conditions, when corticosteroid therapy fails, plasma exchange, cyclophosphamide and IV immunogLOBulin have been shown to be helpful. Future studies on the role of corticosteroids or other immunomodulators in ES should incorporate valid evidence from their use in other conditions involving severe brain damage.

We also found evidence in the systematic review and in the clinical study that several drugs used in ES management are potentially associated with dangerous iatrogenic complications, especially when used in combination. The intemperate or excessive use of diazepam and phenobarbital can easily aggravate respiratory distress and precipitate coma. Quinine, furosemide, mannitol, adrenaline and, to a lesser extent, antibiotic usage were associated with a high mortality in our clinical study. Although this may simply reflect the fact that patients receiving these drugs were in a more critical condition, quinine, mannitol and furosemide can increase or promote cardiovascular arrhythmias, hypoglycemia and fluid and electrolyte imbalance. The use of
these drugs can be optimized by restricting their indication to clearly defined situations where solid evidence of malaria, cerebral edema, pulmonary edema, shock or bacterial infection is available, and careful clinical monitoring is possible.

The patient nutritional status is frequently cited and traditionally considered as having a significant impact on the risk for ES. However, evidence from a recently completed adequately powered large trial (IMPAMEL) using the body mass index (BMI) as an indicator of malnutrition does not confirm this hypothesis. In our clinical study neither the general status nor the BMI was correlated with ES or death from ES. Malnutrition, which causes different degrees and types of immunosuppression, could interfere in complex ways with the immune response that triggers ES. On the other hand, it seems reasonable to think that severe malnutrition can act in synergy with other factors to render the patient more prone to die from ES. Nutritional aspects, including the subtle and difficult to diagnose forms of micronutrient imbalance, need to be better assessed in late stage HAT patients in order to correctly determine the impact and cost effectiveness of currently used multivitamin, iron and food supplementation on ES incidence and mortality.

ES is a severe clinical situation and constitutes a medical emergency. Measures to control ES have to be introduced quickly and effectively to offer the patient the best chances to survive. Diagnosis and management of ES patients requires sufficient medical skills and minimal access to medical equipment and consumables. The systematic review and the findings of the clinical study indicate that there is room for improvement in the management ES patient, even in the frequently severely limited logistic conditions found in most of the HAT treatment centres.

Clinicians can be trained, using a simple ophtalmoscope, to perform optic fundi examination for the diagnosis of increased ICP. An estimation of the clinical signs of fluid overload and deficit can be obtained by bedside
techniques for evaluation of the central venous pressure and by establishing a fluid input-output chart. This fluid balance monitoring would allow a better prevention and treatment of dehydration, hypotension, brain edema and iatrogenic pulmonary edema. In the clinical study, a nasogastric tube, a urethral catheter and an intravenous peripheric access were available in a significant proportion of ES patients. Taking full advantage of the possibilities offered by these simple measures in patient monitoring requires however additional training and optimization of the technical capacity of the HAT treatment centre staff. The usefulness of these capacity-building activities expands beyond the context of ES and could constitute a valuable improvement in the overall performance of the medical and paramedical staff allocated to HAT treatment centres.

16 Perspectives
The combined results of the dual approach used in this work to clarify the etiology, the pathogenesis, the definition, the diagnosis, the existence and weight of risk factors and the role of interventions for prevention and treatment of ES show, first of all, that additional research is needed to obtain robust, reliable and clinically useful evidence on many of these aspects. The highly complex interactions between the trypanosome, melarsoprol and humans and the multiplicity of interventions used for the prevention and management of ES result in many confounders being present in the determination of risk factors and in the role of interventions. Correctly designed and sufficiently powered studies are urgently needed. The results of our combined approach to the problem of ES show furthermore that it is ethically valid to perform them, even if this means abandoning traditionally accepted concepts and interventions. Insufficiently powered studies can only bring more questionable data and speculation on the role of the different variables on ES, which are obviously not needed. The questionnaire developed and used in our clinical study performed correctly for the prospective acquisition of detailed clinical data in the field. With limited additional clinical training and adaptation, this tool may be systematically used
in HAT treatment centres to allow a standardized collection of clinical data. Subsequently, reasonably homogeneous data coming from different centres could be pooled together to obtain a large enough population size that would confer the necessary statistical power to address insufficiently defined statistical trends.

A few statistically valid and biologically sound clinical risk factors for ES and ES mortality emerged from the combined systematic review / clinical study approach. The impact of the several interventions presently used in the prevention and therapy of ES was found to be dubious, but potential ways of optimizing them were obtained. ES is a frequently lethal complication and even if the etiology of the phenomenon is unclear, clinicians still need guidelines to help then diagnose and manage ES. The present work offers the basis for a rational discussion of those guidelines.

Undisputable evidence on the role of the complex factors involved in the etiology and pathogenesis of ES will ultimately come from CNS anatomopathological studies. Presently, autopsies are mostly not performed in the African countries where HAT occurs. Although for cultural and logistic reasons this situation is not expected to change in the near future, an alternative approach consists in applying the method used to explore the pathogenesis of cerebral malaria and to confirm the cause of death in suspected human rabies, i.e. closed-skull cerebral needle biopsy using the supra-orbital foramen technique, in patients how die from ES. This approach, in conjunction with the sophisticated immune histopathological and molecular methods presently available, could bring valuable information on the nature of ES.

CNS imaging using CT scan and MRI of the CNS and polysomnographic recordings also offer the possibility of improving the definition, diagnosis and management of ES. Since CT scan and MRI are becoming increasingly available in a few African cities and the method for polysomnographic
recordings is now applicable in the field, these methods could be used in a research setting to acquire useful information on ES and HAT.

The capillary blood sample collection method onto filter paper cards used in the clinical study proved to be effective and convenient, avoiding the need for blood manipulation and of a cold chain for sample preservation. When used in conjunction with the presently existing molecular methods for genetic analyses in humans, this sample collection method constitutes a performing tool that can be applied to future studies on the genetic basis of ES susceptibility. Knowledge resulting from these studies on ES is potentially useful to also gain additional insight into the complex etiology and pathogenesis of late stage HAT and of similar encephalopathic conditions such as ADEM or AHLE.

HAT is a neglected disease. Since little drug research is going on and given the still high efficacy of melarsoprol in most of the geographical settings where Gambiense and Rhodesiense HAT exists, melarsoprol, in monotherapy or in combination with other existing drugs, will probably still be part of the chemotherapy of sleeping sickness for many years to come. From the proposed model for the etiology and pathogenesis of ES, it seems probable that even lower total doses of melarsoprol will not entirely preclude the development of ES in susceptible patients and that the potential to trigger ES is not an exclusivity of the arsenic compound. Additionally, if the potential of melarsoprol as an anticancer drug in humans is clinically confirmed, we may anticipate that ES will also limit the drug’s efficacy in these conditions. Solving the enigma of the encephalopathic syndrome is a necessity.

The present HAT epidemiological situation provides the possibility of implementing correctly designed clinical studies to clarify many pending issues in sleeping sickness, including those related to our deficient knowledge of many critical aspects of ES. Our dual approach to the problem of the encephalopathic syndrome in HAT identified and described the existing gaps
in that knowledge and pointed the way for future basic and applied research
needs. The methodology developed and used in the clinical study shows
promising potential for application in future field studies. Although needing
further confirmation, as usual in genetic association studies, our original
finding of a significant association between the HLA complex and ES opens
new perspectives for the determination of the molecular basis of ES.
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APPENDICES

APPENDICE 1: CASE REPORT FORM, FRENCH VERSION

APPENDICE 2: INFORMED CONSENT FORM, PORTUGUESE VERSION