Sleeping sickness

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Abstract

Human African trypanosomiasis (HAT), or sleeping sickness, is a vector-borne disease that flourishes in impoverished, rural parts of sub-Saharan Africa. It is caused by infection with the protozoan parasite Trypanosoma brucei and is transmitted by tsetse flies of the genus Glossina. The majority of cases are caused by T. b. gambiense, which gives rise to the chronic, anthroponotic endemic disease in Western and Central Africa. Infection with T. b. rhodesiense leads to the acute, zoonotic form of Eastern and Southern Africa. The parasites live and multiply extracellularly in the blood and tissue fluids of their human host. They have elaborated a variety of strategies for invading hosts, to escape the immune system and to take advantage of host growth factors. HAT is a challenging and deadly disease owing to its complex epidemiology and clinical presentation and, if left untreated, can result in high death rates. As one of the most neglected tropical diseases, HAT is characterized by the limited availability of safe and cost-effective control tools. No vaccine against HAT is available, and the toxicity of existing old and cumbersome drugs precludes the adoption of control strategies based on preventive chemotherapy. As a result, the keystones of interventions against sleeping sickness are active and passive case-finding for early detection of cases followed by treatment, vector control and animal reservoir management. New methods to diagnose and treat patients and to control transmission by the tsetse fly are needed to achieve the goal of global elimination of the disease.

Keywords: Human African trypanosomiasis, review, sleeping sickness, Trypanosoma brucei gambiense, Trypanosoma brucei rhodesiense, tsetse

Epidemiology: Distribution and Trends

The occurrence of HAT is restricted to the distribution of its vector, the tsetse fly, which is exclusively found in sub-Saharan Africa between 14°N and 20°S. The disease has a focal distribution, although there are areas where suitable habitats for the tsetse fly are found but HAT is not. Species and sub-species of tsetse flies are separated into three groups that show different abilities for transmission in humans [3]. More than 250 discrete active sleeping sickness foci are recognized, most of which are in poor and remote
rural areas where health systems are often weak. However, the disease has also been reported in peri-urban areas. Sleeping sickness develops in areas from the size of a village to an entire district [4]. Although the present number of cases seems negligible on a worldwide scale, the characteristics and focal distribution of the disease can have a great socioeconomic effect in the affected areas [5]. Characteristics of transmission of the disease report the distribution of tsetse fly microhabitats along with the occurrence of contacts between people and vectors during activities such as farming, fishing or hunting. In that sense, displacement of populations, war and poverty are factors leading to increased transmission [5]. In West Africa, recent demographic evolution and climatic dynamics have had an impact on the pathogenic system of HAT. Hence, the northern tsetse distribution limit has shifted towards the south, probably because of a decrease in rainfall combined with the impact of human pressure [6]. Sleeping sickness foci have also shifted from the savannah areas to the forest and mangrove areas of West Africa [7]. Concurrently, some tsetse species, such as Glossina palpalis, have adapted to high human densities and are found in the biggest urban centres of West Africa [8].

The aetiological agents of HAT are tsetse-transmitted protozoa of the species Trypanosoma brucei. Two sub-species are responsible for syndromes of markedly different epidemiology and geographical range. Trypanosoma brucei gambiense is mainly transmitted to humans by tsetse flies of the Glossina palpalis group and has almost exclusively a human reservoir, although pigs and some wild animal species have been reported as being smaller reservoirs. It gives rise to chronic, anthroponotic endemic disease in Western and Central Africa. Infection with Trypanosoma brucei rhodesiense is transmitted by tsetse flies of the Glossina morsitans group and involves humans and a substantial reservoir of ungulate wildlife (mainly antelopes) and cattle. It leads to the acute, zoonotic form of Eastern and Southern Africa and tends to occur in the form of epidemic outbreaks.

In 2010, T. b. gambiense was focally endemic in 24 countries of Western and Central Africa (mainly Angola, Democratic Republic of the Congo, Central African Republic, Chad, Republic of the Congo, Côte d’Ivoire, Guinea, southern Sudan and northwest Uganda) and caused > 90% of reported HAT cases, whereas T. b. rhodesiense disease was endemic in 13 countries of Eastern and Southern Africa (mainly Malawi, Tanzania, southeast and central Uganda) and contributed < 5% [1]. The classic geographic separation of the two forms of disease is jeopardized by the continued spread of T. b. rhodesiense in Uganda towards the northwest [9]. Sporadic cases have been reported linked to infection with species that were previously considered non-human-pathogenic trypanosomes, e.g. Trypanosoma evansi [3,10].

In the late nineteenth century, Africa experienced several sleeping sickness epidemics, the most devastating of which was an epidemic with 300 000–500 000 deaths between 1896 and 1906 that mainly affected the Congo Basin and the Busoga focus in Uganda and Kenya. A second major epidemic occurred between 1920 and the late 1940s, which persuaded the various colonial administrations to invest in vector control and mobile teams to undertake active surveillance of the population; these two strategies are still the pillars of control [11]. The disease was almost eliminated in the mid-1960s, followed by resurgence in the late 1990s. This re-emergence was closely linked to a collapse of surveillance and control activities in most endemic countries in the post-independence period, and was further exacerbated by civil conflicts in the region [6,11]. However, a fall in the number of cases has been noted in recent years. Between 1999 and 2009, increased control activities have been associated with a drop in the number of new reported cases in 2009 to below 10 000 for the first time in 50 years, although many cases certainly remain undiagnosed or unreported because of a lack of specificity of clinical presentation or their occurrence in remote or unstable areas, i.e. in areas without surveillance [1,4,12]. WHO [13] used a ratio of 1 : 3 to 1 : 4 to calculate the figure of 50 000–70 000 new cases in 2004. A new HAT atlas initiative has led to the creation of a geographic database to store and update HAT epidemiological data allowing the geolocation of autochthonous cases that have been detected through active and passive surveillance (Fig. 1) [9].

**Parasite and Host Response**

Trypanosomes are unicellular organisms that belong to the family Trypanosomatidae and the genus Trypanosoma. In the initial phase of infection, trypanosomes are restricted to the lymph and blood systems. At a later stage they are also seen in brain parenchyma and cerebrospinal fluid (CSF), but are generally extracellular. Major modifications of the immune system have been observed in HAT. These exacerbated immune reactions do not lead to protection and are involved in immunopathology disorders. Trypanosomes are surrounded by a surface coat composed of a variable surface glycoprotein that protects them from lytic factors in human plasma and is associated with escape to immune reactions. When infection occurs, this glycoprotein is recognized by the host’s immune system, triggering the production of IgM and IgG antibodies. In HAT, an important feature is a dramatic increase in IgM levels, including trypanosome-specific
antibodies and non-specific immunoglobulin production induced by cytokine activation of B cells. Specific antibodies neutralize the corresponding trypanosomes, leading to a decrease of parasitaemia. However, a subset of these trypanosomes change their surface coats to a new variant surface glycoprotein type that is not affected by the circulating antibodies, leading to renewed proliferation until new antibodies are produced. This sequence is repeated, and the immune system is therefore unable to eliminate the parasites [14].

Considering the high degree of antigenic variation of the surface glycoprotein, development of a vaccine is unlikely to be feasible. Some of these antibodies are also raised against auto-antigens, corresponding to aberrant non-specific polyclonal activation of B cells producing natural auto-antibodies and also to antigen-driven antibodies induced by molecular mimicry [15]. Furthermore, the variant surface glycoprotein is associated with major cytokine network dysfunctions. These various elements contribute to collateral tissue damage and immunosuppression. Moreover, trypanosomes have learnt to use immune mechanisms to their own profit beyond alternative macrophage activation, including host arginase induction that leads to the production of the essential parasite growth factor L-ornithine and a depletion of L-arginine, the substrate of nitric oxide (NO) synthase, resulting in lower levels of cytotoxic NO (Fig. 2) [16,17].

Clinical Features and Morbidity

The hallmark of the disease is that it evolves in two distinct successive stages. The first stage is the haemolymphatic stage, which is defined by the restriction of trypanosomes to the blood and the lymph systems. The second stage is the meningo-encephalitic stage, which is characterized by the active invasion of the central nervous system. If left untreated, sleeping sickness caused by either of the two parasites leads to coma and death. In addition, T. b. gambiense infection is characterized by a chronic progressive course with an average duration of around 3 years that can be mistaken for a chronic haemopathy condition. In contrast,
*T. b. rhodesiense* disease presents usually presents as an acute febrile septico-pyaemia-like illness with poor demarkation between stages and leading to death within months [18].

After the tsetse bite, a chancre is rarely seen with *T. b. gambiense*, (Fig. 3) but occurs and can be numerous in 20% of patients infected with *T. b. rhodesiense*. It represents the initial lesion at the bite site, and is characterized by local erythema, oedema, heat, tenderness and a lack of any suppuration.

The leading signs and symptoms of the first stage generally reported in *T. b. gambiense* infection, are chronic and intermittent fever, headache, severe pruritus with scratching, skin lesions, mobile or rubbery lymphadenopathies, oedema of the face and extremities and, to a lesser extent, splenomegaly or hepatomegaly. Palpation of the sub-clavicular and cervical regions (Winterbottom sign) is part of the field diagnostic procedure. Skin eruptions or trypanides are occasionally observed. Cardiovascular alterations are less prominent in the *T. b. gambiense* infection and the early occurrence of myocarditis is considered a pejorative prognostic event [19]. Minor neurological and endocrine disorders reveal the early central nervous system involvement. Deep hyperaesthesia is often reported (Kerandel’s sign).

The clinical signs of second-stage HAT are neuro-psychiatric and endocrinal disorders. Sleep disorder accounts for dysregulation of the circadian rhythm of the sleep–wake
cycle and a fragmentation of the sleeping pattern rather than the frequently reported inversion of sleep [20]. The neurological symptoms include confusion, tremor, fasciculations, general motor weakness, hemiparesis, akinesia or dyskinesia, sensory disturbances with diffuse hyperpathia, abnormal movements and speech disorders. Abnormal archaic reflexes can also arise (palm-mental reflex, sucking reflex). Psychiatric symptoms can dominate the clinical picture and may constitute the first manifestation of the disease. [21]. Endocrine disorders of the thyroid and adrenocortical function can take the form of hypofunction or hyperfunction. A great diversity of clinical pictures can be seen in patients infected with T. b. gambiense, suggesting the presence of distinct genetic groups of trypanosomes [22–24].

At the terminal phase of the disease, central nervous system demyelination and atrophy are accompanied by disturbances in consciousness with progressive dementia. The patient dies in a state of cachexia and opportunistic infections. Mainly during the late stage, stigmatization of patients is common. Neurological and psychiatric sequelae are frequent.

Diagnosis

**Trypanosoma brucei gambiense** human African trypanosomiasis

Diagnosis of T. b. gambiense HAT relies on a three-step pathway: (i) screening; (ii) parasitological confirmation; and (iii) staging [25,26]. The Card Agglutination Test for Trypanosomiasis (CATT) is a rapid serological screening test used in the vast majority of HAT control programmes [27]. The CATT sensitivity on undiluted whole blood is high (87–98%), which leads to a high negative predictive value of a negative test result, as the prevalence of HAT in the tested population usually lies below 5%. This low pre-test probability also explains why all individuals with a positive CATT sensitivity on undiluted whole blood or positive CATT with undiluted or mildly diluted (CATT 1:4) serum require parasitological confirmation, despite a reported 95% specificity. The treatment of serologically suspect patients with a high CATT titre (≥ 1:16) is only acceptable in areas of high disease prevalence (>1%), especially when the most sensitive blood concentration techniques (see below) are not available [28,29]. Accurate serological tests also exist in immunofluorescence or ELISA formats, but they are mainly in use in non-endemic countries.

Parasitological confirmation relies on the microscopic search for trypanosomes in the inoculation chancre smear or cervical lymph node fluid (sampled by puncture), or in the blood. As circulating parasites are few, it is necessary to use blood concentration techniques. Examination of wet and thick blood smears notoriously lacks sensitivity. The microhaematocrit centrifugation technique, or capillary tube centrifugation technique, remains widely used [30]. The microhaematocrit centrifugation technique sensitivity increases with the number of capillary tubes examined (six to eight tubes should be used) but does not exceed 60%. Microscopical reading of microhaematocrit centrifugation technique can be made difficult by the concomitant presence of microfilaria. The quantitative buffy coat is more sensitive, allows for easier discrimination between trypanosomes and white blood cells, and concomitantly detects malaria parasites [31]. Whereas quantitative buffy coat remains in use in some laboratories, it is not used in the field because of its relative sophistication and irregular availability. The miniature-anion-exchange centrifugation technique, which consists of separating the trypanosomes from venous blood and concentrating them in a collecting tube by centrifugation, is currently the most sensitive technique for trypanosome detection [32]. Production of this test, which has been hectic during the last decades, is currently assured by the Institut National de Recherche Biomédicale in Kinshasa, Democratic Republic of the Congo, in collaboration with the Institute of Tropical Medicine, Antwerp, Belgium [33]. Despite its relatively high cost (2–3 €/test), the addition of miniature-anion-exchange centrifugation technique in diagnostic algorithms has been shown to be cost-effective [34]. Detection of parasite nucleic acids by PCR may be a more sensitive approach, but further standardization and diagnostic validation are needed.

Staging by CSF examination after lumbar puncture is mandatory in all patients with confirmed HAT because treatment substantially differs between the first and second stages. Second-stage HAT is defined by the presence of trypanosomes and/or > 5 white blood cells/μL in the CSF. Some controversy exists about the group of patients with 6–10 cells/μL regarding the presence of neuroinflammation and the equivocal outcomes produced by the use of the first-stage disease drug pentamidine. High IgM concentration in CSF is also a reliable marker of neurological involvement [35].

Overall, diagnostic tools and algorithms are complex for use in the context of rural sub-Saharan Africa (Fig. 4), and currently preclude the re-integration of HAT activities within public health services outside reference centres. It is therefore urgent to develop more practical and accurate diagnostic tests.

**Trypanosoma brucei rhodesiense** human African trypanosomiasis

The diagnosis of T. b. rhodesiense HAT follows the same general principles (screening, diagnostic confirmation and stag-
ing) but differs from that for \( T. \) \( \text{b. gambiense} \) in several ways: (i) in the absence of serological screening test for \( T. \) \( \text{b. rhodesiense} \), screening is based on the recognition of non-specific clinical features (e.g., fever) and history of exposure (e.g., tsetse fly bite during a safari in East Africa); (ii) parasitological confirmation is easier because of the high density of blood circulating trypanosomes; and (iii) biological indices, such as coagulation tests, haemoglobin and platelet counts, are more frequently and deeply altered.

**Treatment**

Treatment of HAT, which has been recently reviewed by Burri [36], includes specific anti-trypanosomal drugs, on which we will focus, and concomitant conditions such as bacterial infections (e.g., pneumonia), malaria, severe anaemia, dehydration and malnutrition (Table 1). All anti-trypanosomal drugs are currently donated to WHO by Bayer Leverkusen, Germany and sanofi-aventis Paris, France. Drugs can be ordered from WHO (contact: simarrop@who.int).

**Trypanosoma brucei gambiense** human African trypanosomiasis

Treatment for first-stage patients has relied on pentamidine for several decades. Pentamidine is administered intramuscularly or as an intravenous slow infusion for 7 days. The most frequent adverse effects are pain at the injection site (rarely complicated by aseptic or septic abscess), hypoglycaemia and hypotension. Patients are given sweet food or drinks before

### TABLE 1. Anti-trypanosomal treatment for first- and second-stage human African trypanosomiasis

<table>
<thead>
<tr>
<th>Disease and stage</th>
<th>First-line treatment</th>
<th>Dosing</th>
<th>Other treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trypanosoma brucei gambiense</em> HAT</td>
<td>Pentamidine isethionate</td>
<td>4 mg/kg/day IM or IV (diluted in saline and given in 2-h infusions) x 7 days</td>
<td>Efflornithine: 400 mg/kg/day IV in 4 infusions x 14 days</td>
</tr>
<tr>
<td>First-stage</td>
<td></td>
<td></td>
<td>Second line (e.g., treatment of relapse): Melarsoprol 2.2 mg/kg/day IV x 10 days</td>
</tr>
<tr>
<td>Second-stage</td>
<td>NECT: Efflornithine + Nifurtimox</td>
<td>Efflornithine: 400 mg/kg/day IV in 2 (1-h) infusions x 7 days Nifurtimox: 15 mg/kg/day PO in 3 doses x 10 days</td>
<td></td>
</tr>
<tr>
<td><em>Trypanosoma brucei rhodesiense</em> HAT</td>
<td>Suramin</td>
<td>Test dose of 4–5 mg/kg IV (day 1), then 20 mg/kg IV weekly x 5 weeks (maximal dose/infusion: 1 g)</td>
<td>Three series of 3.6 mg/kg/day IV x 3 days spaced by intervals of 7 days</td>
</tr>
<tr>
<td>First-stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second-stage</td>
<td>Melarsoprol</td>
<td>2.2 mg/kg/day IV x 10 days</td>
<td></td>
</tr>
</tbody>
</table>

HAT, human African trypanosomiasis; IM, intramuscular; IV, intravenous; NECT, Nifurtimox-efflornithine combination therapy; PO, per os.
and are advised to lie down for at least 1 h after injection. Severe complications, such as anaphylaxis and clinical pancreatitis, very rarely occur.

Melarsoprol, an arsenic-based derivative, was the only available treatment for second-stage T. b. gambiense HAT for more than 50 years. It is a poorly tolerated drug with a wide-range of adverse effects such as an encephalopathic syndrome (5–10% frequency with around 50% case-fatality rate), peripheral neuropathy, hepatic toxicity, skin rash, acute phlebitis and vein sclerosis. Moreover, high melarsoprol failure rates have been reported from several endemic countries. Twenty years after the first demonstration of efficacy, eflornithine (α-difluoromethylornithine or DFMO) has finally become available for field use since 2000, and was confirmed to be safer than melarsoprol [37,38]. Eflornithine has gradually replaced melarsoprol as the first-line treatment but universal use has been constrained by the huge requirements in logistics and nursing care (56 intravenous infusions of > 30 min over 14 days). The provision of kits by WHO including all the necessary ancillary materials mitigated some of the difficulties. Efforts to shorten and simplify eflornithine-based therapy have recently been successful, with the completion of a multicentre clinical trial that demonstrated that a nifurtimox–eflornithine combination therapy (NECT) was not inferior compared with the standard eflornithine treatment [39]. NECT was included in the WHO’s Essential Medicines List in May 2009 and a phase IIIb trial (NECT-FIELD) conducted by the Drugs for Neglected Diseases initiative (DNDi) with over 600 patients is underway in the Democratic Republic of the Congo. Médecins sans Frontières has used NECT as first-line treatment in several endemic countries since January 2010. Out of 341 patients with second-stage disease treated in northeastern Democratic Republic of the Congo by Médecins sans Frontières in 2010, only one patient died (0.3%) during hospitalization (Chappuis F, unpublished data).

Trypanosoma brucei rhodesiense human African trypanosomiasis

Suramin is used for first-stage T. b. rhodesiense disease with a complex dose regimen that last up to 30 days. Nephrotoxicity, peripheral neuropathy and bone marrow toxicity are known adverse effects but are usually mild and reversible. Rare acute hypersensitivity reactions can occur, explaining why a low test dose is generally applied before treatment.

Unfortunately, T. b. rhodesiense is innately resistant to eflornithine. Therefore, melarsoprol remains the only effective treatment for patients with second-stage disease despite its disastrous safety profile. The abridged treatment schedule of 10 daily injections, initially developed for T. b. gambiense HAT (IMPAMEL I and II), has recently showed similar safety and efficacy profiles as a prolonged discontinuous regimen (IMPAMEL III study) [40,41].

Post-treatment follow-up

Follow-up should be proposed for all HAT patients after treatment, e.g. 6-monthly for 2 years. As adherence to follow-up in endemic areas is usually low, shortening the follow-up period would be desirable. A recent study showed that most patients can be accurately classified 6 months post-treatment as cured (no trypanosomes and ≤ 5 white blood cells/μL in CSF) or relapsed (trypanosomes or > 50 white blood cells/μL in CSF) with the remaining patients classified at 12 months using a 20 white blood cells/μL threshold [42].

Human African Trypanosomiasis Outside Africa

Imported HAT caused by T. b. gambiense has occasionally been reported in short-term travellers [35,43], but is more often encountered in migrants and expatriates residing in rural or coastal foci [44]. Because of its long incubation period and indolent course, it may remain unrecognized for years [45]. Moreover, for a long time it may be misdiagnosed as a chronic haematological condition. Such undue delay may lead to diagnosis of the disease at an advanced stage (Malvy D, unpublished data). In addition, infected migrants to western countries from endemic foci may be misdiagnosed as psychiatric patients when there is an unrecognized or unrevealed history of past exposure [21]. By contrast, HAT caused by T. b. rhodesiense is more likely to be seen in tourists returning from East African game parks, mainly in Tanzania or Uganda [46–50]. Some emerging tourist destinations (Botswana, Rwanda, Kenya, Zambia and Malawi) are known foci and may pose a risk for travellers [51,52]. Of note, the manifestations of T. b. gambiense and T. b. rhodesiense HAT in travellers appear more similar than in patients from endemic countries, with prominence of acute febrile disease with trypanosomal chancre (Fig. 3) and rash, and severe haematological or electrolyte disturbances [43,45,53]. The only relevant preventive measure for sleeping sickness in travellers is the avoidance of tsetse fly bites. Tsetse flies are attracted to dark colours, particularly blue and black, and to the motion of vehicles. Tsetse flies are capable of biting through loose weave fabrics and are less affected by many insect repellents. In travellers, personal preventive measures may comprise travelling in cars with screened/closed windows, use of insect repellent containing at least 30% N,N-diethyl-3-
methylbenzamidine (or DEET), and the use of insecticide-treated thick-weave clothing that is khaki or olive in colour [54]. Finally, in individuals bitten by a tsetse fly, a close monitoring of the bite site and body temperature for 3 weeks is warranted. Transmission following accidental self-inoculation in a laboratory worker has been exceptionally reported [55].

Towards the Goal of Elimination

Even though some areas at risk are not covered by control and surveillance programmes, most foci of sleeping sickness are historical and well known [56]. Theoretical conditions for transmission break down seem to be obvious with regard to the constraints related to the biological cycle of HAT, at least for T. b. gambiense infection in which animals are not a substantial reservoir. Herein, transmission through the vector is complex and slow, with < 0.1% of flies carrying mature parasites, and the prevalence of infected individuals is usually low. Infected persons can initially remain asymptomatic for long periods, acting as a silent reservoir. Hence, the most important control measure for T. b. gambiense disease is active case-finding by mobile teams followed by treatment of all infected patients [1,3]. Passive screening at health-care facilities for the T. b. gambiense and T. b. rhodesiense infections is also crucial.

In addition, vector control by use of tsetse fly traps or screens helps to reduce fly density [57]. In some areas, such vector control activities are mainly implemented within the Pan-African Tsetse and Trypanosomiasis Eradication Campaign of the African Union. This goal is achieved through an integrated strategy approach that combines insecticide spraying, traps and the very-high-cost sterile insect technique, although others argue for the use of simple technologies such as traps in combination with insecticides [58,59]. In regions where T. b. rhodesiense is endemic, the main challenge is to control the animal reservoir that represents the risk of permanent transmission and unexpected epidemics. Here, although a restricted application of insecticides to cattle (especially belly and legs) represents a cost-effective accessible method of vector control, the control of the disease in wildlife and the vector in protected areas and game reserves could be more complicated, because of conservationist, ecological and environmental considerations [1].

Following World Health Assembly resolutions 50.36 in 1997 and 56.7 in 2003, WHO committed itself to support HAT-endemic countries in their efforts to remove the disease as a public health problem. Concurrently, HAT initiatives and public–private partnerships are currently developing new methods to diagnose and treat patients and to control transmission. The successful development and implementation of these improved tools, and the sustainable access of all populations at risk to surveillance and control activities, are the remaining obstacles to reaching the objective of global HAT elimination.

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Author Contributions

DM designed the article and drafted the sections on parasite, clinical features and control. FC drafted the diagnostic and treatment sections. Both authors reviewed the whole manuscript and approved the final version.

Transparency Declaration

Conflicts of interest: nothing to declare.

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