Human African trypanosomiasis

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Summary

- Difference between Gambian (western) and Rhodesian (eastern) trypanosomiasis
- Restricted to well defined regions in Africa, determined by tsetse fly vectors
- Early/first stage: transient sore, fever, oedema, lymphadenopathy, splenomegaly
- Late/second stage: central nervous system symptoms with abnormal CSF (elevated cells and protein, Mott cells, trypanosomes)
- Diagnosis: always try to detect the parasite
- Repeated thick smears, Buffy coat, Woo technique, mAECT, lymph node aspiration
- When parasite found in blood or lymph node, always lumbar puncture to determine the stage
- Indirect: serology (CATT for West African form), clinical evidence
- Difficult treatment depending on species and stage: pentamidine, suramin, melarsoprol, eflornitine and nifurtimox
- Currently nifurtimox-eflornithine combination therapy (NECT) in first-line against second-stage *T. b. gambiense* trypanosomiasis. Fexinidazole oral short course.
- Importance of early diagnosis and follow-up as well as integration of control in primary care.

General

Human African trypanosomiasis (HAT) is caused by infection with a unicellular parasite. There are two subspecies of these parasites: the West African or *Trypanosoma brucei gambiense* and the East African or *T. brucei rhodesiense*. They cannot be differentiated from each other on morphological grounds. *T. brucei gambiense* has two subtypes, *T. brucei gambiense* type 1 and 2. The main difference resides in their ability to avoid the uptake or (*T. b gambiense* type 1) or to neutralize/compensate (*T. b. gambiense* type 2) the trypanosome lytic factor, a human serum component. *T. b. gambiense* type 2 resembles *T. b. brucei,* an animal infecting trypanosomeand causes a more acute disease than type 1 *T. b. gambiense*.

Transmission takes place through the bite of an infected tsetse fly (Diptera, genus *Glossina*). Since the parasites are transmitted via tsetse saliva, they are also known as “salivaria”, as opposed to *Trypanosoma cruzi*, which belongs to the “stercoraria” because of its transmission via the feces of the kissing bug. In exceptional cases, mechanical transmission takes place via other biting flies (tabanids). Congenital infections are rare. Sexual transmission seems to be extremely rare.

Epidemiology

African trypanosomiasis occurs exclusively in sub-Saharan Africa, with its distribution being defined by the tsetse fly occurrence Because of its clinical presentation, the West African form is also called sleeping sickness. The area of distribution lies between 14° north of the Equator and 29° south of the Equator. The areas of distribution of West African and East African trypanosomiasis show little overlap. Most of the endemic countries have only one form of the disease: the Western form, or the Eastern form. This facilitates national therapeutic guidelines. However, both West and East African trypanosomiasis exist in Uganda. Both forms have their own foci, but these are now converging in Uganda. They did not overlap in 2015, but are separated now by only a narrow corridor of about 100 km. If the transmission areas meet (as feared), it would considerably complicate diagnosis and guidelines for management of clinical cases in Uganda.

There have been several large epidemics in Africa in the last 120 years. One from 1896 till 1906 mostly in Uganda and the Congo Basin. Numbers were skyrocketing in many African countries in 1920 but by the mid-1960s, the disease was under control with less than 5000 cases reported in the whole continent, thanks to mobile teams which carried out the screening of millions of people at risk. After this success, surveillance was relaxed, and the disease reappeared, reaching epidemic proportions in several regions by 1970. In 1998, almost 40,000 cases were reported, but estimates were that 300,000 cases were undiagnosed and therefore untreated. In the last decades of the 20th century, prevalence reached 50% in several villages in Angola, the Democratic Republic of the Congo, and South Sudan. Sleeping sickness was the first or second greatest cause of mortality in those communities, even ahead of HIV/AIDS.

The efforts of WHO, national control programmes, bilateral cooperation and nongovernmental organizations (NGOs) during the 1990s and early 21st century reversed the curve. In 2009, after continued control efforts, the number of cases reported dropped below 10,000 (9 878) for the first time in 50 years. This decline in number of cases has continued with 997 new cases reported in 2018, the lowest level since the start of systematic global data-collection 80 years ago. The estimated population at risk today is 65 million people. The area reporting ≥ 1 case/10,000 inhabitants/year in the five-year period (2012–2016) has shrunk by 61% from the baseline period (2000–2004). Since the number of new human African trypanosomiasis (HAT) cases reported between 2000 and 2018 dropped by 95%, the WHO neglected tropical diseases road map targeted its elimination as a public health problem (< 1 case/10,000 inhabitants/year) by 2020 and interruption of transmission (zero cases) for 2030.

To achieve complete elimination of HAT, the main challenge is to set up a cost-effective, adapted and

sustained HAT control and surveillance strategies. Integration of the vertical HAT control activities in the general health system will be needed, which is often particularly difficult in those peripheral rural areas where the disease is more entrenched and the health system is weak. Sustained commitment of donors will be crucial. The role human asymptomatic carriers, of parasites in the skin, and by the possible animal reservoirs in gambiense HAT epidemiology, will be essential.

Countries reporting cases (year 2019):

T. b. gambiense: Guinea, Equatorial Guinea, Nigeria, Cameroon, Gabon, Chad, Central African Republic, Congo, DR Congo, Angola, South Sudan, Uganda. Countries with historical *T. b. gambiense* HAT with surveillance activities not reporting any cases are Benin, Ivory Coast, Mali, Niger, Senegal, Sierra Leone and Togo. This anthroponotic subspecies affects mainly humans but is sometimes isolated in pigs, dogs, … The role of animals in transmission is unknown but probably very limited.

Between 1999 and 2019, the reported number of new cases of the chronic form of human West African trypanosomiasis (*T. b. gambiense*) fell by 97%, from 27 862 to 864. Importantly, the number of health facilities providing gambiense HAT diagnosis and treatment keeps increasing. Therefore, it can be considered that the observed trends are very likely to reflect a real abatement in disease transmission, despite the challenges always posed by under-detection. Notwithstanding the encouraging indicators, surveillance has weakened in South Sudan and the Central African Republic due to security constraints. So, the risk of deceleration is real and can have serious consequences as was already painfully experienced in the history of HAT.

T. b. rhodesiense : Uganda, Tanzania, Zambia, Malawi, Zimbabwe. No more cases reported from Burundi, Ethiopia, Kenya, Mozambique and Rwanda. This subspecies is a zoonosis affecting both wild animals and domestic cattle. Humans are sporadically infected “by accident”. Contrary to West African HAT, the zoonotic nature of rhodesiense HAT does not presently allow to envisage complete interruption of its transmission.

From 1999 till 2009 , the number of newly reported cases of the acute form of human East African trypanosomiasis (*T. b. rhodesiense*) fell by 81% from 619 to 116. Of note, in 2018 only 24 cases of East African HAT where reported. In Malawi the reported cases rose from 15 in 2018 to 91 in 2019. In contrast with the West African HAT, surveillance has weakened in countries as Tanzania, Uganda, Zambia and Zimbabwe. The replacement of microscopic examination for malaria diagnosis by rapid serological tests now prevents the accidental diagnosis of rhodesiense HAT when testing for malaria. This is exacerbated by a concomitant decrease in HAT-skilled staff who could maintain knowledge and awareness of the disease. Opposed to gambiense HAT, very few innovative tools have been

developed for rhodesiense HAT screening, diagnosis and treatment. These factors in combination with the acute clinical progression of rhodesiense HAT usually prevalent in remote rural areas, are likely to result in non-negligible under-detection. An indirect indication of this under-detection is the fact that 8 cases (6% of the total rhodesiense HAT caseload) were diagnosed in non-endemic countries among returning tourists in 2015–2016.

Trypanosomiasis does occur in South America, but Chagas' disease which is caused by *Trypanosoma cruzi* is clinically very different from African sleeping sickness. There are rare human infections with trypanosomes in India and Malaysia. They were due to accidental zoonotic infections with *Trypanosoma lewisi*, a rat and other rodents parasite transmitted by fleas, or *T. evansi*, a parasite mechanically transmitted by hematophagous biting flies and infecting mainly horses and camels but also buffalo and cattle. A number of human infections with *T. vivax* and *T. congolense* have also been reported. Such infections are very exceptional.

Surra

Trypanosoma evansi causes disease ("surra") in certain animals, such as camels, llamas, horses, buffalo, cattle, dogs, sheep and goats. There is considerable variation in the pathogenicity of different strains and the susceptibility of different host species. The disease ranges from inducing a subclinical infection, mild disease, chronic to overt forms (months to years) and rapid fatal infections (esp. in horses and camels). Deer, capybara and coati can become infected and ill and may also constitute a reservoir. Animals subjected to stress such as malnutrition, pregnancy, work, are more susceptible to disease. Suramin is the most frequently used drug for treatment of surra in horses. Successful treatment by a single dose of diminazene diaceturate has been reported in dogs.

Trypanosoma congolense is the main trypanosome infecting cattle, causing animal African trypanosomiasis (AAT) Every year AAT is responsible for more than 3 million deaths in cattle with estimated annual agriculture economic losses of more than US$ 4.5 billion dollars, making AAT one of the major constrains for sustainable livestock production in Africa. A few indigenous African cow breeds, such as the N'dama breed, tolerate the parasite's presence remarkably well. However, these trypanotolerant animals are not popular with farmers because they grow slowly and are small. Many farmers prefer Boran cattle, which are more beefy with high resistance to heat and ticks but susceptible to AAT.

Trypanosoma equiperdum causes a chronic sexual transmitted disease ("dourine") in horses,

mules and donkeys. Infections are endemic in Eastern and Southern Africa, South America, Mongolia, Russia and Kyrgyzstan. *T. equiperdum* is the only trypanosome that is not transmitted by an insect vector.

Parasite

In general, among trypanosomes, one can distinguish several morphological forms based on the relative position of the kinetoplast to the nucleus. Extracellular African trypanosomes have two main morphologies:

1. Epimastigote: fusiform 20-40 µm long with an anterior placed kinetoplast, in front of the nucleus i.e. on the same side as the flagella is pointing. This stage occurs in the tsetse fly.

2. Trypomastigote: the kinetoplast is located behind the nucleus. The parasites are pleomorphic in human blood. Some are elongated and slender ("slender trypomastigotes") and others are shorter and stumpy. Reproduction in man occurs via longitudinal binary cleavage every 7 hours.

In intracellular trypanosomes, like *T. cruzi* (see Chagas' disease) the amastigote stage is present inside the cell. This multiplication stage is characterised by a spherical form without flagella.

Parasite information

The parasite has only one nucleus, is elongated, contains a giant mitochondrion and has a single flagellum. At the base of the flagellum is the basal body. This lies adjacent to the kinetoplast. The latter is a compact DNA (deoxyribonucleic acid) structure, located in the very long mitochondrion. This mitochondrion is almost as long as the entire trypanosome. The name of the Order to which the parasite belongs – Kinetoplastida – refers to this organelle. Between the basal body and the flagellum there is an undulating membrane which is required for motility. In the form of the parasite such as it occurs in man (trypomastigote), the kinetoplast lies in a posterior position and the flagellum points towards the front, rather like a bowsprit on a large sailing vessel. The parasite occurs in the salivary glands of the tsetse fly as an epimastigote (kinetoplast located just in front of the nucleus).

The genome of *T. brucei* was sequenced and published in Science in July 2005. The DNA in the kinetoplast (kDNA) stains like that of the nucleus (recognizable on a smear). The structure of the DNA in this kinetoplast is very complex. There are numerous (about 40) large DNA loops

("maxicircles") and even more (some 5,000-10,000) small DNA loops ("minicircles").

While in the human host, the parasites are diploid. The parasites replicate in humans by asexual mitosis. Diploid and polyploid forms can be found in tsetse flies. Experimental arguments for meiosis and a possible sexual reproduction in *T. brucei* were first proposed in 1986. In the laboratory tsetse flies were infected with 2 different clones after which hybrid parasites were isolated, which indicates exchange of genetic material. This could be important for a better understanding of the natural parasite populations, e.g. via the various iso-enzyme patterns that occur in nature. Even if these laboratory data were confirmed, it remains an open question how important this is in nature.

The notion of **antigenic variation** in African trypanosomes has been around for a long time. Early investigators would isolate trypanosomes and serum from an animal early in the course of an infection and then again later during the same infection. Early antiserum would kill the initial strain of trypanosome, but did not affect the trypanosome strain isolated later in the infection. It was apparent that the trypanosome population changed over time. When the parasite is present in an individual it is covered with a thick monotonous layer of a single type of glycoprotein, VSG (Variant Surface Glycoprotein). The VSG coat is approximately 20% of total cell protein and includes more than 10 million molecules thus has a vast repertoire of surface antigens. The *T. brucei* genome has around 2000 distinct VSG genes but only one single VSG is expressed at a time. The entire VSG surface of a trypanosome is recycled every seven minutes by a process of VSG endocytosis and exocytosis. When the parasite is transferred to the tsetse fly, the VSG coating disappears within 4 hours and is replaced by an invariant glycoprotein ("procycline" or PARP (procyclic acidic repetitive protein)). After the parasite has completed its cycle in the fly, colonizes the salivary glands and transforms into the metacyclic infectious stage the VSG coating reappears. The metacyclic VSG coat is different from the bloodstream VSG coat having only 12 to 20 VSG types. The metacyclic VSG coat is supposed to limit the first immune response and thus facilitating the parasite establishment and proliferation in the vertebrate host, making the VSG coating of vital importance for the parasite. This explains why only metacyclic trypanosomes (the mature forms in the salivary glands of the insect) are infectious. When an antigenically homogeneous population of parasites is in the human body, antibodies against the VSG of this population are produced. The immune system lyses the parasites which is accompanied by fever. Infections with trypanosomes would be cured quickly, if the parasite population could not constantly change its surface antigens. The switch of VSGs happens about once every 100 cell divisions.

Most of these VSG genes are located on specialized telomeric region defined as the expression site

with around 80% of these telomeres residing on minichromosomes in the nucleus of the parasite. The parasite also has about twenty chromosomes of “normal” size. These do not condense during mitosis. At any one time, only one VSG gene per parasite is active. After destruction of the first dominant population by the immune system, the heterologous parasites increase in number until the variant VSG has induced antibodies and a new cycle of destruction begins. A third population of minority variants then emerges. Antigenic variation is a very important factor in the development of the disease and explains various symptoms (including its chronic course, fluctuating parasitemia and fever episodes).

Vector

Tsetse flies (*Glossina sp*.) are blood-sucking insects that occur only in sub-Saharan Africa and the Gisan oasis in Saudi Arabia. Four different species of fossil flies were discovered in 20-million-year-old mudrock in Colorado, USA, indicating that the insects once existed in North America. The name tsetse descends from the Tswana language. This name was also used by the Matabele and Zulus and refers to the sound that the insects make. An English reporter in Southern Africa at the end of the 19th century adopted this name when he wrote about a fly which attacked horses and cows.

The insects have prominent elongated mouthparts (proboscis), which explains their scientific generic name (“glossus”: tongue). Tsetse flies have typical wing veins, with a “hatchet cell” in the middle. When resting they fold their wings over their back like a closed pair of scissors. Other flies hold their wings more to the side. There are 31 species and subspecies, but less than half are vectors of human trypanosomiasis. The genus *Glossina* is now divided into three subgenera:

1. The fusca-group (subgenus Austenina): not important in human pathology.

2. The palpalis-group (subgenus Nemorhina): these flies prefer dense vegetation in humid areas (e.g. on riverbanks, gallery forests). Their habitat should have exactly the right conditions of humidity, warmth and light intensity, and there should be a blood supply (nearby animals or humans). Humans are frequently bitten when working/standing close to the water’s edge. The flies can also be found in cocoa, coffee, mango and banana plantations; this group is the vector of human West African trypanosomiasis and nagana (= animal trypanosomiasis).

3. The morsitans-group (subgenus Glossina) are distributed over the East African savanna and are zoophilic. They are the vector of East African trypanosomiasis.

Tsetse flies and their bloody bites

As obligate blood feeders, both male and female tsetse flies feed with blood every 3 to4 days. After landing on a host, the fly will lower its proboscis to a vertical position and stab with a rocking motion of the body. The rough dentate part of the proboscis saws through the tissues. The proboscis penetrates the skin while the teeth lacerate the capillaries walls and saliva is injected forming a small pool under the skin. The blood is actively pumped up and stored in the crop for a short time and is then passed to the midgut. Tsetse blood feeding implies manipulation of the host haemostasis, possible by producing and injecting a potent saliva anticoagulant cocktail at the biting site. Until now, two key molecules have been identified to facilitate tsetse blood feeding: the tsetse thrombin inhibitor with anticoagulant activities and the 5'Nuc apyrase with a dual role in platelet activation and aggregation. Bites of forest flies are less painful than those of savanna species. During a bite, tsetse can injects with the saliva infectious metacyclic trypanosomes. Feeding time ranges from 20 to 25 seconds. In a single meal 5-80 mg (max 155 mg) of blood in taken up . A hungry fly can take up a bloodmeal greater than its fasting weight. When satiated, the fly heads to a roosting site to digest at leisure.

Tsetse flies live a few months. If parasites are taken up by a bloodmeal, 99% of the parasites die in the insect's stomach (midgut), but some transform in the procyclic (midgut) and later into the metacyclic trypomastigotes (salivary glands) The tsetse fly becomes infective 2 to 3 weeks after an infective bloodmeal.

Adult tsetse flies are airborne for short periods and rest for the remaining time. On average, they cover 200-300 meters in the dry season. In savanna areas they only take flight at times of the day when temperature is suitable. At the hottest time of the day (above 35°C) and during the night they rest. Farmers take advantage of this trait by driving their herds through infested areas after dusk. In forested areas where temperature swings are less marked they fly more often. There are several different species of tsetse flies, each with its own ecological preference. In an endemic area usually less than 1% of the flies are infected.

Congenital transmission is possible and there are case reports of laboratory accidents, blood transfusion and organ transplantation as transmission route, but they are extremely rare.

Clinical aspects

Infection by *T.b. gambiense*

Any bite from a tsetse fly, whether infected or not produces a local reaction. When the bite is infected a small local wound can appear after 1 or more weeks, but in general after 5-15 days (trypanosomal chancre or sore or trypanoma). This often remains unnoticed in the local population, though it can sometimes reach quite substantial dimensions (2-5 cm). In infected Europeans it is described at a frequency of 25-40%. It involves a central blister or ulcer surrounded by red infiltrated skin. The lesion tends to be minimal painful. When it has healed after 1-3 weeks a depigmented scar can remain. The infection develops slowly if there is no medical intervention. The patient's condition gradually deteriorates, ultimately leading to his/her death in sometimes as short as a few months, sometimes much later. There are two quite artificially separated stages: a preliminary hematolymphatic stage and a second stage with symptoms of meningo-encephalitis. The boundary between these two stages is determined by the findings in the cerebrospinal fluid. The distinction is important for treatment. Asymptomatic human carriers (and spontaneous cure) are described but is rare.

Hematolymphatic stage (first or early stage)

The hematolymphatic stage lasts 6 to 12 months, but sometimes much longer. It is characterized by intermittent unpredictable bouts of fever separated by irregular intervals of days to a month or even more, headache and general malaise. The lymph nodes swell, especially those in the neck (Winterbottom sign). These glands are soft, mobile and not painful. In early West African trypanosomiasis, swollen posterior cervical lymph nodes are found in 50-85% of early stage patients and in fewer than 25% in the late stage. Oedema sometimes occurs (face), as well as pruritus (itching) and transient red spots or a circinate rash (trypanides). This rash can be seen without difficulty on a white skin (reported in 50%) but is difficult to see on a dark skin. The liver and certainly the spleen can be enlarged. There is moderate to severe anemia. Neurological disorders (personality changes), increased sensitivity to pain, especially deep hyperesthesia ("Kerandel sign") can already be present in the first stage. This condition gradually evolves into increasing neurological collapse, characteristic of the meningo-encephalitic stage.

The condition is characterized by a chronic course with flare-ups and quieter periods. These flare-ups are to be interpreted as destruction of the trypanosomes, followed by the development of a new population of parasites carrying a different surface antigen. Lysis of the parasites releases large quantities of antigen into the bloodstream. These form immune complexes with circulating antibodies which then precipitate resulting in perivascular inflammatory symptoms (including vasodilation with increased vascular permeability and oedema). Successive generations of parasites each have a different glycoprotein on the outer membrane. It is to this outer membrane that the antibodies attach

themselves. Whenever a new glycoprotein emerges, the immune system always has to start again from scratch, with the production of new antibodies. This explains the pronounced increase in the immune globulins (especially IgM) in blood and cerebrospinal fluid. The high IgM serum concentration thus results from chronic polyclonal B cell stimulation. Aspecific cross-reacting and auto-antibodies can also be produced, making serological diagnosis of other diseases more difficult. Meanwhile time goes on and the infection worsens.

Meningo-encephalitic stage (second or late stage)

If left untreated, the meningo-encephalitic stage will progress to death in 6 months to 2 years after neurologic symptoms arise. Personality changes increase and the patient usually loses interest in their surroundings. Psychosis sometimes occurs. The patient develops tremor, paresthesia, increased sensitivity to pain, gait disorders, speech difficulties and reversal of the diurnal wake/sleep rhythm. Ataxic dyskinesia is present in most patients. Basal ganglia involvement can produce clinical features which overlap with those of Parkinson's disease. Weight loss and endocrine abnormalities with e.g. impotence are common. Damage to the hypothalamus (paraventricular and supraoptic nuclei) may lead to disturbance of the normal sleep pattern. The patient progressively deteriorates and develops stupor (sleeping sickness!). The patient can still be woken up but will quickly go "back to sleep" again. Daytime sleeping, insomnia and behaviour change are reported in 40%, 55% and 30% of cases respectively. This is finally followed by coma and the patient dies of malnutrition, concomitant infections, accidents and destruction of the central nervous system. This disease is not to be confused with neurosyphilis, tuberculosis, AIDS with cerebral toxoplasmosis or cryptococcal meningitis, alcoholism or schizophrenia.

Histopathological changes include leukoencephalitis with demyelination and accentuation of the periventricular areas. There is a characteristic infiltration of lymphocytes and plasma cells around cerebral blood vessels (perivascular cuffing).

Infection by *T.b. rhodesiense*

Infection with *T. b. rhodesiense* evolves much faster than West African trypanosomiasis. The incubation phase is shorter (1 to 3 weeks). An inoculation chancre often occurs (in traveller this is almost always present), and appears some days before the onset of pyrexia. There is high fever and most patients have signs of multiorgan failure. Hepatitis leads to jaundice, elevated liver enzymes and coagulation disturbances. Myocarditis is common and often gives diffuse T-wave inversions. Heart failure can occur. ARDS can be detected on chest X-ray. Encephalitis leads to neurological symptoms, such as confusion and stupor. Daytime sleeping, insomnia and behaviour change are reported in 75%,

65% and 20% of cases respectively. There is usually no obvious lymph node swelling, but splenomegaly is frequent. The disease evolves to a fatal outcome within a few weeks or months.

Diagnosis

Detection of parasites

In the peripheral blood there is usually a normal white blood cell count (no leukocytosis or leukopenia), a normal platelet count and a slight normocytic anemia. The erythrocyte sedimentation rate is quite high, in part explained by the high immunoglobulins. The diagnosis is best made by detection of the parasite. The sensitivity of conventional parasitological techniques is however quite low. The parasite can be found in fluid from the inoculation chancre, blood (direct examination, thin smear, thick smear, buffy coat), lymph node fluid (needle aspiration) or cerebrospinal fluid (lumbar puncture). In a wet blood smear, the motility of the parasites attracts the eye but the sensitivity of the technique is too low. A Giemsa-stained thick blood smear is more sensitive, but parasites are frequently deformed in this preparation and are therefore easily missed. Lymph node aspiration is done with a dry needle. After puncture the needle is left in place for a while and the node is massaged. A syringe is then fitted to the needle and after aspiration the fluid is put on a microscope slide for direct examination (the motile trypanosomes can then be observed). Several samples will often be needed, as the parasites are not present in large numbers and appear in the blood in intermittent waves. Concentration techniques facilitate the diagnosis: Woo technique, buffy coat from a centrifuged microhematocrit tube or quantitative buffy coat test (QBC). In well-equipped laboratories a miniature anion exchange centrifugation technique (mAECT) is used (Lanham or Lumsden method). Such a column contains diethylaminoethyl-cellulose (DEAE-52). The separation of blood cells from trypanosomes depends on a difference in surface charge of the blood cells and the parasites. This charge is pH-dependent (importance of iso-electric point). Blood is mixed with a particular buffer (PSG = Phosphate-Saline-Glucose) and gently layered on top of the column. The blood will penetrate the gel on top of the column and red and white blood cells adhere to the DEAE gel particles. In this buffer, the trypanosomes are at their isoelectric point (=neither positive nor negative charge) so flow through the column. The eluate containing the parasites is collected and centrifuged. The sediment is examined microscopically to determine if parasites are present. The type of buffer and the temperature at which the test is carried out are of very great importance. The more the disease advances the less frequently are trypanosomes found in the blood, though they are then found more often in the cerebrospinal fluid. The parasites can be cultured *in vitro* in a specific medium (KIVI; Kit for in Vitro Isolation). In theory as few as 1 trypanosome can be detected in 5 ml, though in 50% of the tested cases the culture remains sterile.

Comparison of detection thresholds

Fresh blood preparation (10 µl) 6000 trypanosomes/ml

Thick drop (10 µl) 2000 trypanosomes/ml

Buffy coat (70 µl) 600 trypanosomes/ml

QBC (Quantitative Buffy Coat Test) 16 trypanosomes/ml

MAECT (500 µl) usually 100/ml required

PCR (Polymerase Chain Reaction) 10 trypanosomes/ml

KIVI (Kit for In Vitro Isolation) 1 trypanosome per 5 ml.

Serology

Antibodies can be detected serologically. Several techniques (immunofluorescence etc.) have been developed. There are also methods for use in primitive rural conditions. A cheap and practical method is a direct agglutination reaction of trypanosomes on a plastic card, with macroscopic read-off (**CATT = Card Agglutination Test for Trypanosomiasis**), which was developed by the Institute of Tropical Medicine, Antwerp. This is a good screening method for *T. b. gambiense* in most areas. The sensitivity of the CATT test in areas (e.g. Cameroon) with *T. gambiense* strains which do not carry the variable surface antigen LiTat 1.3 is lower. A drop of blood (finger prick) and a drop of reagent that contains blue-colored parasites of a known serotype are mixed on a white plastic card. The card is mechanically shaken for 5 minutes and then immediately read. When the test is positive (presence of antibodies) the trypanosomes agglutinate and form a blue clot. The CATT has no place in the diagnosis of *T. rhodesiense* infections, except in a chronic form of *T. rhodesiense* which exist in Malawi. CATT must not be confused with the CIATT (Card Indirect Agglutination Test for Trypanosomiasis, an antigen-detection test). Another method is to take a blood drop on very small filter papers (confetti) and examine this later in a laboratory. The patient should be called back later if the result is positive. Antigen-detection methods (ELISA) have also been developed, but are not yet in routine use. A problem arises in persons who have a positive serology, but who are asymptomatic and in whom no parasites are found (wait and see with follow-up or treatment with suramin or pentamidine?). After successful treatment the antibodies remain for years. Antibody detection therefore cannot be used for detecting relapse or reinfection. It is hoped that in the future we shall be

able to prove a cure by monitoring reductions in the levels of circulating antigens.

The CATT is designed for mass screening and still requires agitator rotator, electricity, and refrigeration, there is a need for simple and individual point-of-care tests. In 2013, two lateral-flow **rapid diagnostic antibody-detection tests** were developed for *T. brucei gambiense :* the HAT Sero-K-SeT test (Coris, Belgium) and the immunochromatographic HAT-RDT (Standard Diagnostics, Korea), designed for testing on whole blood, with results provided within 15 minutes. The tests contain variant surface glycoproteins (LiTat 1.3 and LiTat 1.5). Clinical field evaluation showed sensitivity and specificity similar to those obtained with the CATT, but with simpler use (no need of electricity and cold chain). In large multicenter prospective studies, sensitivity of both SD HAT-RDTs was found lower than expected (71%–89%) whereas specificity was very high (98%). However, combining any of these RDTs together or with CATT achieved a very high sensitivity. It appears therefore that both these RDTs achieve a diagnostic accuracy equivalent to that of CATT and may be used instead for both mass screening and clinical care, wherever local conditions do not favour the use of CATT.

In *Trypanosoma b. r.* HAT, the parasite load in blood is usually very high at symptom onset, so trypanosomes are relatively easily detected whenever a blood smear is performed, In travellers, diagnosis of *T brucei rhodesiense* HAT has been almost always made by thick and thin blood film examination, often as a surprise finding. It could, however, be missed when in such circumstances malaria diagnosis is limited to RDT. Although antibody based assays exist for *T brucei rhodesiense* HAT, none have been developed in RDT format.

Genomic tests

The first PCR was developed in 1983 by Kary Mullis (Nobel Prize Chemistry 1993). Since then several PCR variants have been developed. Most techniques consist of an amplification step followed by amplicon electrophoresis in agarose gel, but there are other approaches. The sensitivity and specificity largely depend on the DNA sequence targeted by the primers. Preferred genomic sequences are those which are conserved and unique for the parasite and that occur as multiple copies in the genome. Tests based on extra-nuclear minicircle kinetoplast DNA have failed to live up to expectations. With PCR, formal molecular differentiation between *T. brucei gambiense* and *T. brucei rhodesiense* is possible. *T. brucei gambiense*-specific glycoprotein is only present in *T. brucei gambiense*, while the gene encoding the serum-resistance-associated protein (SRA) is only present in *T. brucei rhodesiense*. Both however are single copy genes. The most interesting next-generation diagnostic for active infection by trypanosomatids is the spliced leader RNA (SL-

RNA) detected by PCR. The splice leader is a conserved species specific sequence capping the mature mRNAs.

Clinical diagnosis

A correct diagnosis can sometimes be reached even though parasites cannot be detected. These “clinical cases” are patients from an endemic area, with clinical symptoms of late stage trypanosomiasis and lymphocytes in the cerebrospinal fluid. Such “clinical cases” may amount to no more than 5% of the total number of trypanosomiasis patients.

Diagnosis, IgM in cerebrospinal fluid

Antibodies should if possible be detected in the cerebrospinal fluid. Determining the IgM content in the cerebrospinal fluid can be very difficult or even impossible to carry out in endemic areas and under field conditions. An experimental latex agglutination test for detection of IgM was developed at the Institute of Tropical Medicine, Antwerp, Belgium. Blood-CSF barrier dysfunction is usually absent or mild and occurs in very advanced late-stage disease. It is possible to calculate and plot diagrams of the quotients CSF/serum concentration for IgG, IgA and IgM (demonstration of intrathecal production of antibodies). Especially intrathecal IgM production will be present in late-stage sleeping disease (occurs in 98% of people with leukocyte counts higher than 20/µl). Similar patterns do occasionally occur in Lyme neuroborreliosis, neurosyphilis, mumps meningoencephalitis and in non-Hodgkin lymphoma involving the central nervous system.

Usefulness of the lumbar puncture

A lumbar puncture is important:

1. sometimes in order to make a diagnosis

2. in order to determine the stage (main purpose)

3. in order to monitor therapy

In the 2nd stage the cerebrospinal fluid is characterized by:

- white blood cell count (WBC) > 5 per mm3, (normally <3)
- protein > 45 mg% (normally 15-45 mg%)

- IgM increase (difficult to carry out; Latex IgM)
- sometimes trypanosomes and/or Mott cells (= degenerated plasma cells: multiple varied size spherical inclusions/ Russell bodies within a plasma cell having an eccentrically placed clock face nucleus; also called morula cells of Mott (in Latin ‘morus’ means mulberry).

Treatment

There are several different treatment schemes that are determined by the vertical control program that is (or was) in place in many areas. The specific therapy is not simple. Drugs that do not penetrate into the cerebrospinal fluid and the brain are useful in the early stage only (prior to invasion of the central nervous system). Drugs that do penetrate the blood-brain barrier must be used in the late stage. Although recent progress has been made, there is an urgent need for less toxic, easy to administrate and cheaper drugs.

Here under are summarized the current guidelines for HAT treatment, according to the causal species and disease stage. Thereafter, each trypanocidal drug is described one by one for information.

Treatment summary

***T. b. gambiense* early stage:**

Pentamidine 4 mg /kg/day IM or IV for 7 days (preferred)

Or suramin test dose 5 mg/kg, then 20 mg/kg/day (max. 1 g) IV on days 3, 10, 17, 24, 31 (alternative)

***T. b. gambiense* late stage**:

NECT: eflornithine 200 mg/kg IV 2 times per day for 7 days + nifurtimox 5 mg/kg/day TID orally for 10 days

Or elflornithine 100 mg/kg IV 4 times per day for 14 days

NB: Melarsoprolol is not used any more for *T.b. gambiense* except in the very rare situations of treatment failure with NECT, and after expert advice. The regimen is then similar to the treatment of rhodesiense HAT late stage (see below: 2.2 mg/kg/day for 10 days with prednisolone throughout the whole period)

T. b. rhodesiense* early stage*:

Suramin test dose of 100 mg (check urine for protein and cylinders), then 20 mg/kg/day (max 1 g) on day 1, 3, 7, 14 and 21 (alternative 20 mg/kg weekly for 5 weeks)

***T. b. rhodesiense* late stage:**

Melarsoprol 2.2 mg/kg/day for 10 days under cover of prednisolone (see above); the previous cumbersome alternative (3-4 series of 3.6 mg/kg/day IV for 3 days weekly) is being abandoned

NB: The first all-oral short course treatment fexinidazole has been approved in 2020 by WHO as a valid alternative treatment for *T.b. gambiense* early stage AND late stage (but only in children 6 years oor mre, if drug administration with food is directly observed T, and for the late stage if there is no advanced neurological disease nor presence of more tha 100 WBC/µL in CSF examination). Details about the rationale are provided below in the paragraph on fexinidazole.

Trypanocidal drugs

Suramin (Germanine®). The compound was developed in 1920. It is best administered by slow intravenous infusion, as intramuscular administration (10% solution in distilled water) is very painful. The drug is active against both *T.b. gambiense* and *rhodesiense*, but its toxicity restricts its use to the latter pathogen, since a better tolerated alternative (pentamidine) exists for the former. Suramin is excreted extremely slowly by the body. This is important if exfoliative dermatitis develops as a side effect. It can cause substantial proteinuria and a nephrotic syndrome. When a test dose of 100 mg is tolerated well, the daily dose 20 mg/kg (max. 1 g) can be given on days 1, 3, 7, 14 and 21. A urine strip should be performed before each administration, to look for occurrence of proteinuria. In the past 20 mg/kg (max 1g) weekly was given for 5 weeks. Fever sometimes initially occurs due to lysis of trypanosomes. Suramin also kills *Onchocerca volvulus* filaria. Patients with active onchocerciasis can exhibit severe side effects to suramin (cfr. Mazzotti reaction with DEC).

Pentamidine was developed in 1941. It is less active than suramin and not active against *T. b. rhodesiense*. pentamidine exists as an isethionate salt (Pentacarinat®, Pentam®) and must be administered parenterally. Intramuscular injections are painful and therefor slow IV administration is preferred. Rapid IV injection causes acute hypotension. Hypoglycemia can sometimes occur due to release of insulin from the pancreas. Other adverse events include pancreatitis, ventricular arrhythmias, hepatotoxicity and kidney failure. This medicine is also used in pneumocystosis in AIDS patients and the treatment of cutaneous *Leishmania guyanensis*. The recommended field treatment is

4 mg kg/day for one week.

Eflornithine (DFMO, Ornidyl®). Di-fluoro-methyl-ornithine or DFMO was first used for trypanosomiasis in 1985. It is very water soluble. This substance penetrates quite well into the cerebrospinal fluid. A cumbersome IV treatment is however required, divided in 4 administrations per day for 2 weeks. Eflornithine is rather well tolerated, although hematotoxicity is possible (and bacterial infection of IV lines in the tropics) as well as seizures. Unfortunately it is active only against *T. b. gambiense*. In monotherapy, the dosage regimen is 100 mg/kg/6 hours IV x 2 weeks via physiological fluid infusion. Concentrations in cerebrospinal fluid in children seem to be lower than in adults. Children require a higher dose (150 mg/kg 4 times a day). If used in combination with nifurtimox, the dose is 200 mg/kg 2 times a day (over 1 hour) for 7 days.

Nifurtimox (Lampit®): Cf. Chagas' disease. After thorough pharmacokinetic studies, a multicenter trial has evaluated the safety and efficacy of the combination of nifurtimox (oral, for 10 days) with eflornithine (IV, 2 administrations of 200/kg per day for 7 days), compared to eflornithine in monotherapy (100 mg/kg 4 times a day) for 14 days. The nifurtimox-eflornithine combination treatment (NECT) was not inferior to eflornithine (> 95% cure rate) but much easier to administer and cheaper (14 infusions of eflornithine instead of 56!). Further field phase 4 studies confirmed these excellent results. This combination has also the theoretical advantage to prevent emergence of resistance. The NECT has been endorsed by WHO in 2012 and has become the first-line therapy in all endemic countries. The drugs are provided for free by WHO to the national HAT programs.

Melarsoprol (Arsobal®) was developed in 1949. Because of the demonstrated efficacy of eflornithine for second stage *T. b. gambiense* trypanosomiasis (with limited side effects), the use of melarsoprol is nowadays limited to the *T. b. rhodesiense* form (second stage). This trivalent arsenic compound is insoluble in water or alcohol. It is therefore dissolved in propyleneglycol. This solvent is highly irritant to tissues. It causes phlebitis and chemical cellulitis when administered paravenously. Melarsoprol may only be given by very slow IV infusion. It has a significant trypanocidal activity (as can be measured via bioassay) in plasma and cerebrospinal fluid for up to several days after administration, although melarsoprol can then no longer be detected with HPLC (high performance liquid chromatography). The molecule is transformed into biologically active metabolites such as melarsene oxide that irreversibly binds to pyruvate kinase, which disrupts energy production in the parasite. The same effects happen in host cells, rendering the drug highly toxic. Resistance to melarsoprol is described. Toxicity results in polyneuropathy and reactive encephalitis. Encephalopathy tends to manifests itself as a sudden violent neurological deterioration at the end of the first series or during the second series of injections. At present, there seems to be no way to predict which patient will develop encephalopathy. Corticosteroids seem to diminish the risk and severity of the

encephalopathy (controversial). It is therefore imperative to administer prednisolone before using melarsoprol. In general, one can expect lethal reactive encephalopathy after administration of melarsoprol in about 3-5% of cases. Clinically, there are three syndromes of reactive arsenic encephalopathy:

- convulsive status associated with acute cerebral oedema, due to diffuse lesions with hemorrhagic encephalitis.
- rapid progressive coma without convulsions
- acute nonlethal mental disturbances without neurological signs (e.g. psychosis)

Another toxic effect of melarsoprol is polyneuropathy (analogous to heavy metal intoxication). This results in diminished sensitivity and/or paresthesia's in hands and feet, as well as motor signs. In this case melarsoprol should if possible be stopped and vitamin B (e.g. thiamine) administered.

In the past, treatment regimens consisted of 12 injections over a 30-day period, but current regimens with daily administration of 2.2 mg/kg for 10 consecutive days have proven a similar efficacy towards *T.b. rhodesiense* and *gambiense* without increase in toxicity, avoiding very long hospitalizations.

Experimental medications

Fexinidazole is a product related to megazol, tinidazole and metrinidazole. After demonstrations of in vivo trypanicidal activity in 1983 the drug languished in obscurity for more than 25 years. Fexinidazole was "rediscovered" as an oral drug which might be used in early and late stage sleeping disease. In African trypanosomes, the mode of action of nitro drugs involves reductive activation via a NADH (reduced form of nicotinamide adenine dinucleotide)-dependent bacterial-like nitro-reductase. In a randomized controlled trial including 394 patients, treatment success at 18 months was slightly lower with fexinidazole (1800 mg once a day, days 1 to 4; 1200 mg/day, days 5 to 10) than with NECT therapy (91% vs 98%) but this difference was within the predetermined acceptability margin of 13%. The death rates (none directly attributable to treatment) and severe adverse events were similar between the two groups. In a subgroup of patients with > 100 >WBC/µl in the CSF, fexinidazole was inferior to NECT (87% vs 99% cure). Based on these new key findings, the WHO has revised its recommendations in 2020, and considers nowadays fexinidazole as a valid treatment for BOTH stage 1 and stage 2 gambiense HAT, PROVIDED that the patient does not present with advanced neurological disease (and/or > 100 WBC/µL in CSF examination) and that treatment administration can be directly observed (lower efficacy if no concomitant food intake). Implementation of this new strategy is just starting.

In sharp contrast with the gambiense HAT, rhodesiense HAT treatment did not progress at all. Studies to examine the efficacy of fexinidazole against *T. b. rhodesiense* are ongoing. If successful, fexinidazole has the potential to become a safe, efficacious, affordable, oral short-course for both stages of *T. b. gambiense* and *rhodesiense.*

The Drug for Neglected Diseases Initiative (DNDI) has recently developed the compound SCYX-7158, the first oxaborole-based agent (the boron atom being essential for the trypanocidal activity even if the mechanism of action is unknown). Phase 1 studies have been completed. Phase 2/3 study results are expected in 2021. The drug has a very long half-life (17 days in healthy volunteers), making it very promising as a single dose oral treatment for both HAT studies.

Treatment follow-up

After treatment the patient should have a regular follow-up for 2 to 3 years for possible relapse (*T. b. gambiense*: check every 6 months). The first sign of relapse is often an increase in the cell count in the cerebrospinal fluid, followed by a rise in its protein content. Recurring fever, drowsiness and chronic headache are also signs of relapse. Unfortunately, obtaining a CATT negative result after treatment for HAT cannot be relied upon to confirm successful treatment. Further works needs to be done to address this question.

Recent studies have however demonstrated that it was possible to simplify and shorten the period of follow up after treatment for second stage *T. b. gambiense* infection according the following rules:

- At 6 months presence of trypanosomes or WBC > 50 in the CSF are considered as failures; WBC count < 5 in the CSF is considered as a cure (no further lumbar puncture). The remaining patients need to be re-evaluated again at 12 months by lumbar puncture.
- At 12 months: cure if no trypanosomes and CSF WBC < 20; failure if > 20 WBC in CSF or trypanosome.

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