Trypanosomiasis

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Human African trypanosomiasis

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 anno 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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American trypanosomiasis (Chagas' disease)

Summary

- *Trypanosoma cruzi*, only in the New World
- Transmission via bugs, blood transfusion, congenitally and orally (bug feces in food/drink)
- Importance of poverty (housing) in transmission
- Acute (especially children): chancre, Romaña's sign, fever, lymphadenophathy, myocarditis, hepatosplenomegaly
- Chronic: cardiac arrhythmias, heart failure, emboli, apical aneurysms
- Chronic: dysphagia, constipation (mega-syndrome)
- Diagnosis: clinical + thick smear/buffy coat (early), serology, xenodiagnosis, ECG, X-ray (late), PCR
- Treatment in the early phase still reasonably successful with medication; in the late phase difficult and probably useless
- Nifurtimox badly tolerated as a 2 to 4-month treatment; benznidazole: problems with bone marrow toxicity, hypersensitivity, peripheral neuropathy.
- Prevention: much progress in recent years via vector control and control of blood banks.

Introduction

Historical note

In 1907 the physician Carlos Chagas (1879-1934) was working in Lassance, a small poverty-stricken town on the Sao Francisco river in the state of Minas Gerais, Brazil. The town had been built along the railway from Rio de Janeiro to Belem. Chagas treated the workmen for injuries, syphilis, malaria etc. He noticed that cardiac arrhythmias occurred frequently. One day an engineer brought him an insect of the type which was known to often suck the blood of humans at night. Chagas wondered if this creature could also transmit malaria like the Anopheles mosquitoes. In the bug he discovered a unicellular parasite. In April 1908 he found the same parasite in a sick cat. Two weeks later, in the same house, the parasite was found in the blood of a 3-year-old child (Rita), who was ill with fever. Her face, liver, spleen and lymph nodes were swollen and the child died shortly afterwards. In the house there were countless bugs which tested positive for the parasite. He sent bugs to Rio, to Oswaldo Cruz, his former teacher (Brazilian physician 1872-1917). In the laboratory the parasite caused an infection in marmoset monkeys (Callithrix sp.), rodents and puppies. The disease caused by this parasite, American trypanosomiasis, was named after Chagas. The parasite was given the name Trypanosoma cruzi. The parasite did not always trigger

disease, however. In 1908 Chagas also discovered the parasite in another person (Bernice). This woman died in 1989, still infected, but without signs of organ involvement.

The infection apparently already existed before contact with the West. In 1985, 22 mummies were found in the Andes mountains. These were 1500 years old. In approximately half of them the heart, colon and/or oesophagus were clearly enlarged (lesions typical for Chagas' disease). *Trypanosoma cruzi* DNA was found in 1999 in a 4000 year-old mummy in Northern Chile. In one of his books Charles Darwin describes how in 1835 in South America he was bitten by the bugs. It is possible that he incurred infection and later developed a chronic form of the disease.

Distribution

The infection only occurs in America in endemic regions. It is a disease associated directly with poverty. The severity varies from region to region. In the South of Texas there are very few cases. Infections occur in Central America sporadically. Although the disease is endemic in large areas of South America (in particular in the "Gran Chaco" region), the majority of those infected have no symptoms. Until recently it was thought that approximately 16 million persons were infected, but these figures are under review (see Prevention). The disease is transmitted via the faeces of an infected bug.

Reservoir

The parasite, *Trypanosoma cruzi*, occurs in more than 100 species of mammal (opossums, guinea pigs, goats, dogs, cats, rats, mice, and so on). There are several known (and probably also some unknown) subtypes each of which has its own distribution and probably also its own pathogenic features. In view of the extent of the animal reservoir eradication of the parasite will not be possible. This does not mean that the disease and the transmission cannot themselves be controlled. At present the strains are divided into two groups. *Trypanosoma cruzi* I has an extensive sylvatic reservoir, of which opossums appear the most important. This group is not very common in the "Southern Cone" countries (Argentina, Brazil, Chile, Paraguay, Uruguay), but it is virtually the only form which occurs north of the Amazon region. T. cruzi II seems to be chiefly associated with rodents and is common in the Southern Cone.

Transmission

Transmission occurs chiefly **via infected bugs.** These large insects like to bite sleeping humans at night (a mosquito net gives protection). They have a sharp proboscis which at rest is folded below the head like a jack-knife. When biting they inject anticoagulants and an anesthetic substance into the

wound. Since this makes their bite quite painless (kissing bugs), people seldom wake up and several bites may take place unnoticed in the course of one night. The parasite is not inoculated directly by the bite, as Chagas initially thought. In 1913 Brumpt showed that the parasite is found in the faeces of the insect. While the animals suck blood, they defecate. By scratching, a bitten person can bring the faeces into the bite wound or rub them into the conjunctiva. The parasites multiply in humans and then appear in the blood. The cycle is completed when a subsequent bug drinks infected blood. In the bug the parasite undergoes further changes and after 2 to 3 weeks is excreted with the faeces during a subsequent bite. It is estimated that the risk per bite by an infected *Triatoma* is one in a thousand. The existence of **oral transmission** has been suspected for quite a while. It was demonstrated in animals and has now been confirmed in some human cases. How frequent oral transmission happens is not clear yet. Food or drink contaminated with the liquid faeces of infected bugs or containing (crushed) dead bugs may lead to infection not only in experimental animals but also in humans. Small outbreaks of acute Chagas are regularly reported from Northern Brazil in the last years. The parasite could withstand short periods of freezing, but not decontamination with sodium hypochlorite or heating to 80°C. **Congenital infection** (1 to 2 % risk) and transmission via **blood transfusion** also occur (poor people often sell their blood). To give an idea of the scale, this implies for example that several thousands of babies are born with congenital Chagas each year in the USA, and a lesser number in Europe (from immigrant mothers from endemic areas). Transmission via transfusion is particularly important in urban zones and has been reported outside endemic countries. The risk of infection after a contaminated blood transfusion is estimated at one in five. There are sporadic cases of **accidental contamination** of laboratory staff (finger prick, aerosol) and after **organ transplantation** (including in non-endemic countries).

Vectors

The bugs are also known locally as “vinchucas” or “barbeiros”. Of the approximately 120 vector species only about 7 are important. Each species has its own region of distribution:

- Central America and northern South America: *Triatoma dimidiata* and *Rhodnius prolixus*
- South America (south of 5° S): *T. infestans*, *T. braziliensis*, *T. sordida*, *Panstrongylus megistus*

The bugs mentioned here are the main vectors. Other bugs also play a part in different regions. The bugs each have their own preferred biotopes. *T. dimidiata*, for example, is often found inside houses on the floor or the lower 150 cm of the walls or immediately outside in dung heaps, hollow trees, etc. In contrast, *R. prolixus* prefers to live in palm leaves either in the roof of the house or in the tree itself. In and around the house the bugs can feed on animals (e.g. dogs are important because they sleep at night when the bugs are active). The vectors often live in chicken runs but the chickens themselves

are not infected (they do eat bugs). During the day the insects hide in all kinds of cracks and crannies (importance of earthen or adobe walls) and in the roofing (straw, wood, etc). It can be seen immediately that the key word in Chagas' disease is "poverty". These are insects which reproduce slowly and whose geographical spread is slow. Migration of bugs, by migrating birds for example still needs to be studied. In view of these characteristics and the fact that the important vectors live around houses they can easily be reached by eradication campaigns.

A fertilized female lays several hundred eggs in her lifetime. From the egg comes a nymph which always needs a blood meal for its subsequent development stages (both sexes suck blood). The last instar will develop into an adult insect. During a blood meal they suck more than their own weight in blood. This takes 10-25 minutes. The insects may live for up to 2 years (5 years for *T. barberi*). *Rhodnius prolixus* has a relatively short generation time (3-5 months), while for *T. dimidiata* this time is quite long (1 year or longer). Long generation times make the development of resistance to insecticides difficult.

Parasite

In stained blood preparations the parasites are C- or S-shaped with a prominent kinetoplast towards the rear (trypomastigotes). The nucleus is elongated and the undulating membrane is usually not clearly visible. After infection multiplication of the parasite in the human is solely intracellular. They form microscopic pseudocysts in the tissues (similar to toxoplasmosis and sarcocsytosis). This occurs mainly in the heart, muscle cells, some nerve cells and the lymphatic system. In the cell the parasite is small and rounded with no flagellum (amastigote). When the infected cell ruptures, parasites are released into the blood circulation where they become elongated and develop a flagellum. These forms can then infect other cells or be ingested by a bug.

Clinical aspects

Infection and incubation

Incubation period after exposure to vector-borne *T. cruzi* is 1 to 2 weeks, although longer incubation times are sometimes reported. If the parasites penetrate via the conjunctiva, there is unilateral redness and oedema of the upper and lower eyelids after 4 to 12 days. This is "Romaña's sign", named after the Argentinean physician Cecilio Romaña, who described the oedema in 1935. This swelling may last for weeks. Sometimes there is also swelling of the ipsilateral lymph nodes (including the pre-auricular lymph nodes). Trypanosomes may be found in the tears at this stage. If inoculation is in the skin there is local oedema and redness ("chagoma") in 75% of cases. This remains for 1 to 4

months. From these sites the infections spreads.

Acute stage

The incubation period is followed by the acute phase which lasts 4 to 8 weeks. Many infections are initially asymptomatic. Acute symptoms occur more frequently in children than in adults. Dissemination of the parasite from the inoculation site may go unnoticed but may also give rise to acute illness with muscle pain, local or generalized oedema, swollen liver, spleen and lymph nodes. Moderate fever is almost always present in symptomatic cases and may persist for a long time, two or even four months. Sometimes there is also acute inflammation of the heart (myocarditis) with arrhythmias, decreased blood pressure, and heart failure. As with other forms of myocarditis the electrocardiogram is frequently abnormal. There is low QRS-voltage, prolonged PR- and/or QT-interval, T-wave abnormalities. Rarely there are ventricular extrasystoles or atrial fibrillation (the prognosis is poor if this occurs). Acute inflammation of the brain and meninges (meningo-encephalitis) occurs, chiefly in young children. Inflammation of the heart and brain may be fatal. There is pronounced lymphocytosis and monocytosis. The acute-phase case fatality rate is nowadays estimated to be 0.25 to 0.50% with early treatment.

Latent period

If the patient survives the initial phase (which is usually the case), a latent period occurs of indeterminate duration. The patient is asymptomatic, seropositive and the parasitemia is very low. Focal lesions are found in 60% of endomyocardial biopsies from patients in the latent phase. A positive xenodiagnosis can be obtained in 50% to 100% of these patients. For xenodiagnosis 10 to 40 non-infected bugs (e.g. *Dipetalogaster maxima* or *Triatoma infestans*) feed on blood from the patient. The faeces from these animals are investigated after 30, 60 and 90 days. In the event of immunosuppression there may be an acute flare-up, including meningo-encephalitis associated with AIDS or heart transplantation.

Chronic phase

Gradually the patient develops symptoms. These vary greatly from region to region. Lesions of the heart, oesophagus and colon are the most common.

Chronic heart problems

Chronic damage to the heart muscle cells and the cardiac conduction system (including that caused by auto-immune mechanisms) leads to heart failure. There is dyspnoea during exertion, orthopnoea and sometimes paroxysmal nightly dyspnoea, oedema of the feet and ankles, congestion of the neck veins, enlarged liver and crackles over the base of the lungs. Sometimes there is pulsus alternans: the peripheral arterial pulsations are alternately strong and weak. The precise pathophysiological mechanism is not fully known. The apex of the heart, which is normally situated on the mid-clavicular line, is displaced to the left. The heart sometimes becomes enormous, which may lead to clot formation in the heart. If blood clots break loose, there may be embolic complications: cerebrovascular accident (CVA), ischemia of limbs, renal infarction. Apical lesions in the left ventricle (wall thinning, intramural bleeding, aneurysms) are typical and occur in approximately 50% of patients. Unlike arteriosclerotic post-infarction aneurysms, in Chagas' disease the apical cardiac tissue does not consist of scar tissue, the wall is simply thinned. Right ventricular lesions occur in 10 to 20%. Cardiac arrhythmias may cause palpitations, dizziness, syncope and sudden death. On the electrocardiogram a right bundle branch block is often seen, together with a left anterior hemiblock, ventricular extrasystoles, abnormal Q-waves and/or AV-conduction disturbances. The coronary arteries are normal. A complete left bundle branch block is exceptional, unlike in idiopathic dilated cardiomyopathy. Sudden death is common in people with Chagas' disease. Probably this is due to ventricular tachycardia which changes suddenly into ventricular fibrillation.

In advanced heart failure, typical radiographic signs may be observed on a chest X-ray: cardiomegaly, prominent hili and distended pulmonary veins in the upper fields, pleural fluid, interstitial pulmonary oedema (fluid in the interlobular septa with Kerley B lines), peribronchial cuffing and finally alveolar pulmonary oedema ("butterfly oedema").

The degree of heart failure is often indicated using the New York Heart Association classification:

- Grade I : asymptomatic
- Grade II : symptoms only during moderate to severe exertion
- Grade III : symptoms during mild exertion
- Grade IV : symptoms at rest. Patient generally confined to bed/chair.

Oesophagus and colon problems

Due to involvement of the small nerves in the oesophagus and colon, peristalsis is reduced and these organs are distended. This occurs in 5 to 10% of seropositive people south of the Amazon, and is virtually absent further north. *Trypanosoma cruzi I* and *II* are both associated with cardiac lesions, but intestinal lesions only occur in infection with *T. cruzi II* (the southern area).

Mega-oesophagus is characterized by difficulty in swallowing (dysphagia), choking, hiccups and nocturnal cough. This often leads to under-nourishment and loss of weight. Aspiration pneumonia is the most feared complication with substantial mortality. A clinical aid for detecting delayed oesophageal emptying is to measure the time between swallowing a mouthful of water, and observing the abdominal noises (stethoscope on the epigastrium). Normally this is less than 10 seconds. A distended oesophagus may also be shown on X-ray. The parotid gland may hypertrophy and lead to so-called “cat’s face”.

Mega-colon can lead to pronounced constipation, meteorism (abdominal distension), abdominal pain and functional intestinal obstruction due to involvement of the myenteric (Auerbach) plexus and the submucosal (Meissner’s) plexus). The abdomen is often distended. Fecaloma, volvulus and peritonitis are complications.

The nervous system

In no other infectious disease is the involvement of the autonomous nervous system as important as in Chagas’ disease. Denervation of the parasympathetic nervous system is better documented and is much more pronounced than denervation of the sympathetic system. There can be sensorimotor polyneuritis. There is some hypoesthesia and paraesthesia, but chiefly a reduction or loss of tendon reflexes. The EMG is disturbed. In the central nervous system there is meningo-encephalitis in the acute phase, but the abnormalities in the chronic phase need to be better defined. In flare-up (e.g. AIDS) there may be intracranial hypertension, lesions of the cerebral nerves, paresis, plegia, stupor and convulsions. The cerebrospinal fluid exhibits a normal number of cells or pleocytosis with predominant lymphocytes and an elevated protein content. At times *T. cruzi* may even be detected in the cerebrospinal fluid. A CT scan of the brain shows one or more necrotizing lesions which may or may not be ring-shaped, with haemorrhages usually subcortical in the brain hemispheres and occasionally in the cerebellum or the brain stem. *T. cruzi* lesions rarely occur in the basal nuclei. These clinical pictures should be differentiated from cerebral toxoplasmosis, abscesses, mycoses, tuberculomata or other mycobacterial lesions, metastases or lymphoma.

Of all cerebral vascular accidents leading to stroke, about 20% are secondary to embolism from a blood clot secondary to atrial fibrillation. If patients do not take oral anticoagulants, an average of 5% CVA’s per year can be expected, which roughly translates to 50% of patients with CVA within 10 years after onset of atrial fibrillation. However for several reasons (mostly haemorrhagic) 20-40% of patients cannot be treated with oral anticoagulants. Most of the clots (90%) originate when blood stagnates in the left atrial appendage, also known as the left atrial auriculum.

Congenital infection

About 1 to 2 % of babies born to seropositive mothers are infected. They may be asymptomatic (rarely) or may develop hepatosplenomegaly, neurological involvement, myocarditis, oedema and a bleeding tendency. The babies may be dysmature and/or premature. Fever is rare in these children. The mortality may be as high as 50% and they tend to die within a week. Those who survive will generally have permanent residual neurological damage.

Diagnosis

In the acute stage the parasite may be found in the blood via a thin **blood smear**, thick smear or buffy coat. As a concentration technique an anion-exchange minicolumn may be used (Woo's technique similar to Lanham's column, but with a different buffer, see African sleeping sickness). Strout's concentration technique includes the double centrifugation of serum (from 10-20 ml of blood), after which the motile trypanosomes can be detected in the sediment. **PCR techniques** for *T. cruzi* exist, but can only be carried out in better equipped laboratories. The **serology** is positive from the fourth week. To know whether the neonate from a seropositive mother is infected, PCR is performed and IgM antibodies in its blood are determined. A positive serology (IgG) 6 months after birth also indicates infection. In-vitro and in-vivo culture is possible, but usually not available. Biopsies of lymph nodes, heart and muscles sometimes show parasitic pseudocysts (amastigotes in the cells). This is quite an aggressive technique and not very sensitive.
Dipetalogaster maximus is a blood sucking bug which can take up to 4 ml of blood in one meal. It is best known for its use in xenodiagnosis (cfr. supra, latent period) of Chagas' disease.

Following WHO recommendations in patient with latent infection (indeterminate), 2 or 3 different positive serological tests are required before ascertaining the diagnosis of Chagas disease.

Prognosis

In an endemic region an asymptomatic person with positive serology is probably a carrier (xenodiagnosis positive in 50 to 100 % of cases). The percentage of seropositive persons who develop symptoms is highly dependent on the geographical region (e.g. 10 to 30%). Some people have mega-organs but are asymptomatic.

Chagas' disease variables associated with adverse outcome

- 2 points: Male

- 2 points: Low QRS voltage on ECG
- 3 points: Non-sustained ventricular tachycardia on 24-h Holter monitoring (run of 3 or more consecutive VES, with a frequency >100).
- 3 points: Left ventricular systolic dysfunction: segmental or global wall-motion abnormality on echocardiography (quid apical aneurysms, intracavitary thrombus)
- 5 points: NYHA III or IV
- 5 points: Cardiomegaly present on CXR, defined as a cardiothoracic index > 0,5

Results:

- < 6: low risk 14% mortality rate in 10 years
- 7- 11: intermediate risk 44% mortality rate in 10 years
- 12-20: high risk 84% mortality rate in 10 years

Treatment

Acute phase

The acute phase lasts up to 60 days. All patients who are in this phase should be treated.

Congenital infection

All infected children should be treated. The earlier therapy is begun, the better the results.

Chronic phase

Etiological drug treatment is indicated for “recent” chronic infections (a few years). In practice all children younger than 10 years are treated. If mega-oesophagus is already present the dysphagia should be treated (the passage and absorption of oral medication may be severely impeded). Etiological treatment in these latter patients was not advised formerly but more recent data have brought this into question. In a study in Argentina, 131 patients with chronic Chagas’ disease were treated with benznidazole. After an average follow up of 8 years, 4.2% exhibited ECG changes compared to 30% in the untreated group. There was also considerably less clinical deterioration in the treated group (2.1% compared to 17%).

The results of a large multicenter prospective study (the BENEFIT study) has however recently

demonstrated that an etiologic treatment with benznidazole did not provide any clinical benefit when a patient with chronic infection had already developed a cardiomyopathy (no reversibility). Treating this group of chronic patients appear to be futile while exposing them to some drug toxicity. Whether this is also true for a patient with latent infection and no complication (yet) will require additional evaluation. Such studies are however very difficult to conduct due to the very long latency period to obtain robust clinical outcome data.

Accidental infection

This may occur for example in laboratory staff. A serum specimen should be frozen before beginning treatment and a second blood sample taken 4 weeks later. Serology is performed on these paired sera. Benznidazole 7-10 mg/kg/day x 10 days is the usual treatment regimen in this situation.

Transplant patients

There are two possible situations: transplantation of an infected organ into a non-infected patient and transplantation of a healthy organ into an infected patient. A donor may be infected so that the recipient becomes infected. Normally the donor is tested beforehand and positive donors are refused, but nevertheless these situations sometimes occur. Alternatively transplantation may be carried out on a patient who is a chronic carrier. The immune suppression that these patients undergo [steroids, azathioprine (Imuran®), tacrolimus (Prograft®) and cyclosporine (Sandimmun®)], may lead to reactivation of Chagas' disease. In both cases treatment with benznidazole 5 mg/kg/day x 60 days, is indicated.

HIV patients and Chagas

Infection with HIV may lead to significant flare-up of Chagas' disease. In endemic regions all HIV patients should be monitored for Chagas' disease. If positive, benznidazole is recommended. There is insufficient data concerning chemoprophylaxis. Since the initial step is often serology, one would normally first try to confirm the diagnosis with a second serological test (ELISA-based preferably) and by looking for circulating parasites by microscope (QBC or buffy coat), PCR and perhaps xenodiagnosis. If the diagnosis is confirmed, the patients deserve to be treated as their risk of severe complications (cardiac, digestive or CNS) is high. Benznidazole is preferred to nifurtimox, since nifurtimox is a treatment that is really badly tolerated in adults (notably a lot of nasty allergic reactions). Benznidazole 5mg/kg (max 300mg) daily for 60 days is not an easy treatment to administer neither (beware of skin toxicity!).

Pregnancy

Treatment during pregnancy is not recommended, although congenital Chagas has been well documented. It is clear that more understanding and better outcomes are sorely needed. Infants of infected mothers have to be carefully followed-up to early detect congenital Chagas.

Treatment: Drugs

There are several problems. The drugs have an unsatisfactory cure rate. The chronic lesions may be caused by auto-immune mechanisms and might not be improved by eradicating parasites (as suggested by the recent BENEFIT trial). Parasites play however some role since the disease worsens during immune suppression as in transplantation and in HIV. The drugs should be given long term (minimum 2 months). Results vary from country to country, possibly due to a difference in parasite susceptibility. Side effects occur more often in adults than in children. It is best to avoid steroids or other immunosuppressive drugs, since these may exacerbate the infection.

Nifurtimox (Lampit®) 5 mg/kg/day orally, slowly increased to 15 mg/kg/day (divided over 3 doses) for 2 to 4 months. There are regular problems for the sustainability of its production. Side effects: neurotoxicity (insomnia, tremor, polyneuritis), nausea, leukopaenia, thrombocytopaenia or hypersensitivity. May cause haemolysis in G6PD deficiency [glucose-6-phosphate dehydrogenase]. In the acute phase the parasites disappear from the blood in 80 % to almost 100 % of cases. The actual cure rate is 50-60%. In a prospective study conducted in Switzerland among Bolivian immigrants, more than 90% of the patients developed some side-effects, sometimes severe (angioneurotic oedema, Dressler syndrome) and half had to discontinue the drug before the end of the 2-month therapy.

Benznidazole (Radanil®, Ragonil®, Rochagan®) 5-10 mg/kg/day orally for 1 to 2 months. Administration (generally 100 mg tablets) is twice daily. The same side effects as nifurtimox, but less frequent and less pronounced, although skin rash occurs relatively frequently (up to 30-40% of patients, with probably some genetic predisposition) sometimes accompanied by swollen lymph nodes or angioedema. The pharmaceutical company Roche has donated all commercial rights and the technology to manufacture benznidazole to the Brazilian government. In all countries 2-month treatment is recommended, except in Argentina where experts recommend a one-month treatment only.

Other types of drugs for treatment

Posaconazole, an anti-fungal therapy, was found to be inferior (in terms of parasitological failure) to benznidazole in a randomized control trial in Spain, published in 2014.

Fexinidazole has a clear anti-*T. cruzi* in vitro activity, but no clinical study has taken place so far.

Ravuconazole is a new triazole with in vitro activity against species of Candida, Cryptococcus and Aspergillus, but also in vitro and in vivo (mice) activity against *Trypanosoma cruzi*. Ravuconazole has a long half-life in humans, which hopefully will facilitate compliance in patients. Clinical trials for its use in Chagas' disease are ongoing in Bolivia.

In the chronic phase the usefulness of these drugs could not be demonstrated (BENEFIT Trial) at least in patients having already (mostly heart) complications.

Symptomatic therapy is therefore indicated: oesophageal sphincter dilation, extramucosal cardiotomy (Heller's operation), colon surgery. An experimental treatment is the endoscopic injection of botulin toxin into the distal oesophageal sphincter (e.g. 20 U into each quadrant).

In heart failure diuretics, ACE-inhibitors and antiarrhythmic drugs may be beneficial. Beta-blockers are best avoided in view of the AV-conduction problems and brady-arrhythmias. Aspirin or anticoagulants are indicated for patients with atrial fibrillation, previous embolic phenomena and apical aneurysms. Amiodarone (Cordarone®) is effective in more than 50% of patients who develop ventricular extrasystoles or ventricular tachycardia. A bifascicular or trifascicular conduction block, also a second or third degree AV-block are contra-indications. A high incidence of "torsades de pointes" has been observed during use of disopyramide and other class I antiarrhythmic drugs.. Pacemakers, automatic defibrillators and cardiac surgery (including heart transplantation) are reserved in practice for those with financial means and these persons have an inherently low risk of infection. It is obvious that such costly procedures will not be within the financial means of the average Chagas' patient.

Prevention

The animal reservoir of *Trypanosoma cruzi* cannot be eradicated. There is no vaccine. Chagas' disease is typically a disease of poverty. Improvements in housing (brick or plaster walls, corrugated iron roofs, long-acting insecticides on house walls) diminish the insect population. A mosquito net has also proven usefulness here. Serological testing of the blood used for transfusion is very helpful. To

date the various biological methods of eradication of the vectors (insecticide sprays, increasing natural enemies) which have been tested have not been effective because a new ecological balance is very quickly achieved but have brought substantial control in most regions.

In 1991-92 the “Southern Cone Initiative” project was launched by Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay with the objective of stopping the transmission of Chagas’ disease. In 1997 Peru joined the project. After an initial phase for preparation (charting the foci, programming the activities, calculating the costs) there was an attack phase with insecticides, repeated after 3 to 6 months. Insecticide-containing paint is cheaper than the traditional insecticides which are applied by spraying. Insecticides dispersed by fumigant canisters were also used. These are locally produced e.g. in Argentina, are cheap, effective and also active against *Aedes aegypti*, the important dengue vector. At present there are effective colourless long acting insecticides. The fact that people see the bugs, cockroaches, etc. lying dead after spraying is a bonus which makes it easier to accept the spraying procedure. In the Southern Cone Initiative, 1,800,000 houses were treated with pyrethroids (deltametrine, lambda-cyhalotrine, cyflutrine) by the year 2000.

Since then there has been further selective treatment of the houses which still exhibited infestation with triatomes. Simple “sensor boxes” of cardboard (traps for the bugs) were placed in the rooms and the occupants themselves could simply ascertain the presence of triatomes. The last phase is surveillance for the detection of residual foci. This is decentralized and involves the population. The effectiveness of the control program has been demonstrated by the very pronounced drop in seropositivity among young children. The surveillance phase has been reached in 6 countries of the Southern Cone. At present there are several South American countries (Colombia, Ecuador, Venezuela) which have a national control program. Similar programmes were begun in Central America in 1997: Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Mexico and Panama. These programs can only be successful if there is participation of the population and if they can be continued for long enough. The latter is a political decision.

In July 2007 the WHO Global Network for Chagas Disease Elimination was launched in order to coordinate global efforts to eliminate this disease. It includes also many non-endemic countries (such as Spain or USA) where Chagas disease in Latin American immigrants have given rise to a substantial number of secondary transmission (by blood transfusion or transplantation), requiring locally adapted control efforts (screening).

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