UPDATE ON HUMAN AFRICAN TRYPANOSOMIASIS (SLEEPING SICKNESS)

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ABSTRACT

Human African trypanosomiasis (HAT) , also known as sleeping sickness, is one of Africa's 'neglected diseases', and is caused by infection with protozoan parasites of the *Trypanosoma* genus. Transmitted by the bite of the tsetse fly, it puts 70 million people at risk throughout sub-Saharan Africa, and is usually fatal if untreated or inadequately treated. In this brief overview some important recent developments in this disease are outlined. These cover various aspects including a reduction in disease incidence, newly recognised parasite reservoir sites in humans, disease outcome, novel diagnostic methods, new and improved treatment, and disease neuropathogenesis.

Human African trypanosomiasis (HAT), which is also called sleeping sickness, continues as one of Africa's major killer diseases which is greatly feared and occurs in 36 countries throughout sub-Saharan Africa where 70 million people are at risk of the disease (1-3). HAT is caused by protozoan parasites of the genus *Trypanosoma* which are transmitted to the susceptible host via the bite of the blood-sucking tseste fly of the genus *Glossina* (1,2). The two variants of HAT are the West African form caused by *Trypanosoma brucei gambiense* (*T.b.gambiense*) and the East African form caused by *Trypanosoma brucei rhodesiense (T.b.rhodesiense*), resulting in a disease lasting months to years in the former case and an acute disease lasting a few weeks in the latter case (1,3). While *T.b.gambiense* causes about 95-97% of total HAT cases, with *T.b.rhodesiense* causing about 3-5% of all cases, the latter variant constitutes about 18% of the total disease risk and is also causes about two thirds of HAT cases in US and European visitors to the East African safari parks (4,5). In the early (stage 1) haemolymphatic phase of infection after the initial bite of the tsetse fly, the parasites multiply and spread throughout the bloodstream, lymphatic system and systemic organs, following which the parasites cross the blood-brain barrier (BBB) to enter the central nervous system (CNS) causing the late (stage 2) encephalitic phase resulting in a wide variety of neurological symptoms (6). If untreated, or inadequately treated, HAT is almost always fatal. Over the last few years there have been several advances in this field and in this brief updating overview some of these are outlined. Overall, the advances have been in the disease incidence, the parasite reservoir sites in humans, disease outcome, novel diagnostic methods, new and improved treatment, and disease neuropathogenesis.

The precise incidence of HAT has always been difficult to determine and an influential World Health Organisation (WHO) report in 1998 suggested that there were at that time about 300,000 new cases of HAT every year (7). While it is possible that this may have overestimated the true incidence of the disease, which is difficult to determine accurately, the rapid and progressive decline in the annual number of new cases of HAT since then has been impressive. This result has been achieved as a result of the combined and coordinated work of WHO, African governments, non-governmental organisations, charities and other bodies (1). Thus, in 2009 WHO estimated that there were as few as 9878 new HAT cases per year , and by 2014 the number of reported cases was reduced to 3796 cases (with <15,000 estimated new cases) (2,8) , and by 2016 the figure was further reduced to 2184 new cases per year (9). It is therefore not surprising that WHO perceives as realistic goals both the elimination of sleeping sickness as a public health problem by 2020 and the achievement of

interruption of its transmission by 2030 (9). It should be appreciated, however, that HAT caused by *T.b.rhodesiense* will be extremely difficult to eliminate in the future because cattle are the animal reservoirs of the human parasites (as opposed to the human reservoir of the parasites causing *T.b.gambiense*) and mass culling of cattle is neither desirable nor probably achievable . However, by combining vector control to limit the tsetse fly population with isolation and treatment of humans with *T.b.gambiense*, the latter disease may be potentially eliminated.

An important recent finding in this context is the remarkable observation that in humans the skin is a reservoir for the trypanosome parasites (10). This finding of extravascular trypanosomes occurred in skin biopsies from individuals who were undiagnosed and in whom parasites could not be detected in the blood. In experimentally infected mice the authors also found that parasites could be detected in their skin and could even be transmitted to a tsetse fly vector (10). These striking findings call into question the long term goal of disease elimination and may also have great relevance to current diagnosis and treatment.

While HAT is usually a fatal disease in the absence of adequate treatment, more recent studied have indicated that this is not always the case. A small number of infected individuals appear to be tolerant of or resistant to trypanosomal infection without exhibiting any clinical features of disease in an analogous way to certain breeds of cattle such as the Ndama and West African Shorthorn which have been shown to be trypanotolerant with genetically determined resistance to infection (9,11). Thus, a few patients with *T.b.gambiense* infection maintain an asymptomatic state and can be aparasitaemic and also seropositive or even seronegative (12,13). Some of these individuals had either declined treatment or had self-cured, with the latter being regarded as resistant rather than tolerant of infection (12,13). It is currently unknown just how widespread this phenomenon is or whether patients who are tolerant and seropositive pose a risk of infection to others and so should be treated accordingly. Relevant to this is the recent report of a man infected with *T.b.gambiense* who had survived 29 years with the disease (14). Whether prolonged survival with disease is a rarity or more common than had previously been thought remains to be seen.

Diagnosis of HAT should ideally always be established by direct visualisation of the trypanosomes in the blood or lymph nodes when samples are examined under a microscope. While this is usually possible in *T.b.rhodesiense* cases because of the high levels of parasitaemia, this may well not be possible in the case of *T.b.gambiense* infection where the

parasitaemia is cyclical, possibly related to a greater adaptation of the parasite to the host (1,3,11). Therefore serological methods have been used for several years in the latter case. Recently, so-called Rapid Diagnostic Tests (RDTs) such as the SD BIOLINE HAT have been developed which have high specificities and sensitivities, and which use serological techniques to recognise a variety of trypanosome antigens such as different Variable Surface Glycoproteins (VSG) (15,16). These RDTs may supersede the older Card Agglutination Test for Trypanosomiasis (CATT) which is useful as a screening test and especially in regions of high HAT incidence. However it should be appreciated that a positive RDT may not necessarily establish that an infection has occurred as it may just reflect an anti-trypanosomal immune response to a bite by an infected fly, and also it is unable to distinguish between an active trypanosomal infection and a previous exposure to HAT which may have been treated successfully (9). Nevertheless the advent of RDTs represents a very useful addition to the diagnostic armamentarium for HAT.

Diagnostic staging to distinguish between the early and late stage of HAT is also extremely important to achieve, especially in view of the toxic nature of drugs for CNS disease (see below), but unfortunately this has proved very problematic in practice. The WHO criteria for the definition of late-stage (CNS) disease is based on the examination of the cerebrospinal fluid (CSF) by lumbar puncture (LP) and is the presence of >5 White blood cells (WBC)/mm^3 and /or the presence of trypanosomes (1,3). While widely used, this criterion is not generally accepted with different physicians using different CSF WBC criteria to define late stage disease. There is also a dissociation between the biological definition of late stage disease and the criteria used for instigating late stage drugs (3). Although several reports have purported to show novel CSF criteria for defining CNS disease (1,9) these have all been compared to the WHO CSF criteria which certainly do not represent a gold standard. This 'circular argument problem' has persisted and has recently been discussed in detail (17).

While drug therapy for early stage HAT has been effective and only mildly toxic intravenous [IV] suramin for *T.b.rhodesiense* and intramuscular or IV pentamidine for *T.b.gambiense*), the drugs currently used for late stage disease are very toxic (1-3). Melarsoprol is the first line drug for CNS *T.b.rhodesiense* and, while effective, it is very painful to administer and causes a post-treatment reactive encephalopathy (PTRE) in about 10% of cases about half of which are fatal and recent studies indicate a fatality rate of about 6% with this drug (1,2). Melarsoprol is also effective against CNS *T.b.gambiense* but was superseded as first line

therapy by NECT (nifurtimox-eflornithine combination therapy) for this variant (18) , but this drug combination is ineffective against CNS *T.b.rhodesiense* (1). An orally effective drug for late -stage disease that is free of side effects would therefore be of great therapeutic value and might also obviate the requirement for an LP to analyse the CSF. Over the last few years several novel and orally administered drugs for CNS HAT have therefore been developed, or are in the process of development, or are under critical evaluation.

In a randomised phase 2/3 study over a period of 18 months in the DRC and Central African Republic, it was recently reported that the nitroheterocyclic drug oral fexinidazole was successful in treating 91% of patients with late-stage *T.b.gambiense* disease compared with a success rate of 98% of patients who had been treated with NECT (19). The side-effects attributed to the two therapies were similar. This is a significant advance though the European Medicines Agency (EMA) noted that in the subpopulation of patients with a CSF WBC of >100/mm^3 the efficacy of fexinidazole was 86.9% compared with 98.7% in the NECT arm indicating that the risk of failure was greater with fexinidazole in this subgroup (20). For this reason the EMA recommended that HAT patients with CSF WBC of >100/ mm^3 should only be treated with fexinidazole if no other adequate treatment such as NECT is available or tolerated. Further studies with fexinidazole are awaited including the extent to which it may or may not be effective in cases of late-stage HAT due to *T.b.rhodesiense.*

There are also other novel oral drugs in the pipeline. A notable one is a compound called SCYX-7158 which is a member of the oxaborole group. In a mouse model of HAT this drug cured late-stage disease, and was also shown to have a good safety profile in a phase 1 study in humans (21). It is now undergoing evaluation in a phase 2/3 trial in CNS HAT and the results are eagerly awaited. A different approach has been to modify the melarsoprol drug itself to make it less toxic but orally administered and equally efficacious. Melarsoprol cyclodextrin inclusion complexes ('complexed melarsoprol) , which are constructed by incorporating the melarsoprol drug into a cyclodextrin molecule, were shown to be effective in curing late-stage disease in a very well established mouse model of HAT while also being free of over toxic effects (22). This drug now has dual EMA/ US Food and Drug Administration (FDA) designation as an 'orphan drug' for the treatment of African sleeping sickness. The EMA has also approved a trial protocol for a first in man phase 2 trial of this drug in *T.b.rhodesiense* HAT. It is hoped that such a trial can start in the near future.

Recent advances in our understanding of disease neuropathogenesis have been steady but, in my view, incremental rather than dramatic. A crucial tool in this respect has been the use of experimental animal models of early and late stage HAT, in particular the use of a highly reproducible mouse model which closely mirrors the human disease both clinically and neuropathologically (11,23). Two particular, and related, areas of advancement have been the use of modern imaging techniques such as Magnetic Resonance Imaging (MRI) and bioluminescence imaging to better understand the disease, and also a focus on BBB function at various stages of disease progression. Thus MRI can be used to assess the degree of BBB damage, which can be quantified, during experimental trypanosomiasis infection, and this has been shown previously not to correlate with the degree of inflammatory cell infiltration (24,25). Leaking of contrast agent within the BBB, indicating impairment, has also been shown to occur prior to the development of CNS disease , and this BBB damage progresses as the disease advances (24). Bioluminescence imaging has also been applied to experimental HAT infection. For example, it was recently shown that using such non-invasive imaging with intravital multi-photon microscopy and a genetically modified Trypanosoma brucei brucei expressing luciferase, trypanosomes enter the brain meninges as early as day 5 postinfection (26). Such technology should prove useful during the process of drug screening for treating trypanosomiasis infections. The modern notion of an early invasion of trypanosomes into the CNS was also supported in such a murine model in a study using intravital brain imaging where bloodstream forms of *T. b. brucei* and *T. b. rhodesiense* entered the brain parenchyma within hours, before a significant level of microvascular inflammation became detectable and in the absence of overt cerebral injury (27). The significance of such early invasion of the brain by the trypanosomes is not yet determined but it is consistent with the hitherto surprising presence of neurological features being detected during the early-stage of *T. b. rhodesiense* infection in HAT patients in Uganda and Malawi (28). Further, the increasing use of genetically modified trypanosomes with specific alterations is very likely to enhance our knowledge of the evidently complex interaction between host immunological defences and harmful parasite-induced factors, in particular at the level of the BBB.

Conflict of Interest Statement

I declare that I have no conflicts of interest to report

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