The challenge of *Trypanosoma brucei gambiense* sleeping sickness diagnosis outside Africa

Running title: Diagnosis of imported sleeping sickness

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Summary

Sleeping sickness is a lethal African disease caused by parasites of the *Trypanosoma brucei* (*T.b.* ) subspecies, which is transmitted by tsetse flies. Occasionally, patients are reported outside of Africa. Diagnosis of such imported cases can be problematic when the infection is due to *T.b. gambiense*, the chronic form of sleeping sickness found in West and central Africa. The low number of trypanosomes in the blood and the non-specific, variable symptoms make the diagnosis difficult, particularly when the index of suspicion is low. When the trypanosomes have penetrated into the central nervous system, neuropathological signs become apparent but even at this stage, misdiagnosis is frequent. Rapid and correct diagnosis of sleeping sickness can avoid inappropriate or delayed treatment and even death of the patient. In this article, an overview on diagnosis of imported cases of *T.b. gambiense* sleeping sickness is given, and possible pitfalls in the diagnostic process are highlighted. Bioclinical parameters that should raise the suspicion of sleeping sickness in a patient who has been in West or central Africa are discussed. Techniques for diagnosis are reviewed. A clinician suspecting sleeping sickness as import pathology should contact the national reference centre for Tropical Medicine, Centers for Disease Control and Prevention in Atlanta or World Health Organisation in Geneva for clinical consultation and provision of specific diagnostic tests. Appropriate drugs for sleeping sickness treatment are also provided by WHO and CDC.

Keys: *Trypanosoma brucei gambiense*, sleeping sickness, diagnosis, travel medicine,
Introduction

Sleeping sickness, also called human African trypanosomiasis, is caused by *Trypanosoma brucei* (T.b.) *gambiense* or *T. b. rhodesiense* and is transmitted through the bites of tsetse flies. The chronic disease form due to *T. b. gambiense* is endemic in West and central sub-Saharan Africa. The acute disease, due to *T. b. rhodesiense*, is confined to eastern and southern Africa. The actual number of infected persons in Africa is estimated between 300,000 and 500,000. According to WHO (J. Jannin, personal communication), about 20 *T. b. gambiense* and 30 *T. b. rhodesiense* infections are diagnosed yearly outside Africa, but the intense international migration, tourism, peace keeping, and military interventions in areas at risk, in combination with the current sleeping sickness epidemic, might lead to increased numbers of cases outside of Africa in the future. Unlike *T. b. rhodesiense* sleeping sickness, which is characterised by high parasite numbers in the blood and therefore is relatively easy to diagnose, imported *T. b. gambiense* sleeping sickness, often remains unrecognised for years.\(^1\)-\(^8\) The disease is hardly known by Western clinicians and, due to its chronic character, the link with a journey in Africa and the infective bite is often not obvious. This is in contrast with endemic areas, where in some foci active screening for sleeping sickness takes place by specialised mobile teams, and the health workers are aware of the disease and know how to diagnose it.

Correct and rapid diagnosis of the disease is of utmost importance. It not only can prevent death of the patient, but also can reduce the risk of progression of the infection to the late stage, which is more difficult and dangerous to treat than the early stage, and which is associated with a higher risk for neurological sequellae after treatment.

Clinical picture

Clinical signs and symptoms of *T. b. gambiense* trypanosomiasis are largely non-specific, and there is considerable variation in the clinical picture of the disease. Hospitalisation of *T. b. gambiense* patients outside Africa usually occurs either early in infection, after development of fever or a trypanosomal chancre,\(^9\)-\(^11\) or much later in the disease course, after development of serious neurological signs.\(^1\)-\(^3\),\(^5\),\(^7\),\(^8\),\(^12\)-\(^15\)

Unlike in African natives, where the early stage of *T. b. gambiense* infection may pass almost symptomless and remain unnoticed, the onset of trypanosomiasis is almost always acute in individuals from countries not endemic for the disease.\(^14\) Whereas presence of a trypanosomal chancre has
never been reported in Africans, it has been described to occur in a frequency of 25-40% in T.b. gambiense infected Europeans and may be the first sign of infection.\cite{14} The chancre develops after 5 to 15 days at the site of the bite, expanding within a few days from a erythematous papule to a hard and painful nodule, several centimeters in diameter, with central ulceration. Within a few weeks, the chancre disappears without leaving a trace. In virtually all patients, a fever develops 1 to 3 weeks after the infecting tsetse bite. After the intial febrile attack, lasting for maximum one week, periods of fever become irregular, and may be separated by intervals of a few days to a month or more. The observed fever may lead to presumptive anti-malaria treatment.\cite{6,10} The trypanosomal rash, reported to occur in up to half of the patients of European origin, is invisible on a dark skin. It presents as papulo-erythematous eruptions affecting mainly the trunk and shoulders and is evanescent. Pruritus, often associated with skin rash is observed in up to half of the cases. The enlargement of the glands of the posterior cervical and supraclavicular groups may be the key sign of the disease in early stage. Sometimes enlarged neck glands are visible, but usually the neck has to be palpated to discover them. Lymph nodes are painless, rubbery and mobile and can persist over weeks or months.

None of these early clinical features is constant, except fever. Only the trypanosomal chancre, the rash, and posterior cervical lymphadenopathy -if recognized- can be considered characteristic. Observation of one of these early symptoms should, in combination with the relatively recent travel history in endemic areas, point to a possible diagnosis of trypanosomiasis.

After the acute infection, symptoms and signs may subside, and the disease may remain quiescent until invasion of the central nervous system takes place. The occurrence of neurological signs such as sleep disorders, sensory disturbances, endocrine dysfunction, tone and mobility disorders, abnormal movements, mental changes or psychiatric disorders is correlated to the localisation of trypanosomes in the central nervous system.\cite{16,17}

Neuro-imaging data of trypanosomiasis are scarce,\cite{5,7,12,15,18} for obvious reasons. For more detailed descriptions of the clinical presentation of T.b. gambiense sleeping sickness including import cases we refer to reviews of Duggan & Hutchinson,\cite{14} Dumas & Bisser,\cite{16} Edan\cite{19} and Boa\cite{17} and the individual case reports cited above. It should however be kept in mind that due to the polymorphism of clinical signs, sleeping sickness may simulate a great number of other diseases.\cite{16} and that the differential diagnostic question may highly vary in individual patients.
Biological alterations and presence of antibodies

Some standard bioclinical parameters such as anaemia and thrombocytopenia may provide indirect diagnostic evidence for trypanosomiasis. Highly elevated IgM concentrations in blood should direct the diagnostic pathway towards more specific examinations. The IgM concentrations in *T. b. gambiense* patients can be up to 16 times normal, as a result of the polyclonal, non-specific B-cell activation. During the accompanying poly-specific immune response, a variety of non-trypanosome specific antibodies and auto-antibodies are produced e.g. against fibrin, fibrinogen, DNA, red blood cells, thymocyte antigens$^{20,21}$ and CNS components such as myelin, galactocerebrosides and neurofilament$^{22-24}$. Antibodies specific for other pathogens, such as *Toxoplasma gondii*, *Strongyloides stercoralis*,$^{12}$ Epstein Barr Virus, cytomegalovirus,$^{25}$ *Plasmodium fieldi*, *Plasmodium brasiliana* and *Borrelia burgdorferi*,$^2$ have been observed in patients with *T. b. gambiense* sleeping sickness and constitute an additional risk for misdiagnosis.

*T. b. gambiense* specific IgG and IgM antibodies are present in high concentrations and are mainly directed against the immunodominant surface glycoprotein antigens of the parasite. They can be detected by ELISA or immunofluorescence, using purified trypanosomal glycoproteins or whole trypanosomes of selected antigen types.$^{26,27}$ The test of choice when *T. b. gambiense* sleeping sickness is suspected is the card agglutination test for trypanosomiasis (CATT).$^{28}$ It is a simple, 5-minute test that is based on the agglutination of whole, fixed and stained trypanosomes in the presence of specific antibodies. The test is used by almost every control programme in *T. b. gambiense* endemic areas for seroscreening of the population at risk. Due to its ease of application and almost immediate result the CATT is also recommended for use outside Africa in case of trypanosomiasis suspicion.

Trypanosome detection in blood

Although (bio)clinical and serological suspicion might suggest African trypanosomiasis, the infection should always be confirmed by parasite detection before starting the treatment, because of the potentially serious side-effects of antitrypanosomal drugs. However, trypanosomes in *T. b. gambiense* infection can be difficult to detect, as a consequence of the often low parasite load.

Trypanosomes are easy to recognise in a wet blood preparation, because their motility attracts the eye, but sensitivity of the technique for *T. b. gambiense* infections is insufficient. A Giemsa-stained
thick blood smear is more sensitive, but inexperienced laboratory technicians may have difficulty in recognizing parasites because they are frequently deformed in this preparation. The examination of lymph node aspirate also requires experience, and is often negative in the late disease stage.

The use of specialised concentration techniques for trypanosome detection in blood can provide a more straightforward solution. The most sensitive technique, the mini-anion exchange centrifugation technique,\textsuperscript{29} is not widely available. In non-specialised laboratories, the application of the micro-haematocrit centrifugation technique\textsuperscript{30} is an alternative. By centrifugation of blood in a haematocrit centrifuge, trypanosomes are concentrated at the level of the white blood cells, between the plasma and the erythrocytes and can be detected under the microscope. To increase sensitivity, more than one capillary tube can be prepared.

Different experimental methods for trypanosome-specific nucleic acid detection by PCR have been described for the diagnosis of trypanosomiasis,\textsuperscript{8,31-34} but PCR has not yet been fully validated. Problems with its reproducibility, and prolonged positivity after successful treatment have been reported.\textsuperscript{8,34}

Cerebrospinal fluid examination and stage determination

In sleeping sickness, the cerebrospinal fluid (CSF) is usually examined for leukocyte count, total protein concentration and the presence of trypanosomes. These examinations are performed for discrimination between the hemo-lymphatic, first and meningo-encephalitic or second disease stage, which determines the choice of therapy. Pentamidine is used for treatment of hemo-lymphatic stage \textit{T.b. gambiense} trypanosomiasis and is relatively well tolerated. However, it is inefficient in the meningo-encephalitic stage when trypanosomes have invaded the central nervous system since it does not cross the blood-brain barrier. For treatment of second stage sleeping sickness in Africa, melarsoprol, an organo-arsenical drug, is still the first choice. Melarsoprol is highly toxic and can provoke severe adverse reactions, the most feared being an encephalitic syndrome which occurs in 5-10\% of the treated patients and is fatal in half of them.\textsuperscript{35,36}

Second stage trypanosomiasis is diagnosed in patients with a CSF leukocyte count of more than 5 cells/μl or presence of trypanosomes in CSF. The latter can be detected in the cell counting chamber, or by double or simple centrifugation of the CSF.\textsuperscript{37,38} In Africa, total protein determination in CSF is only rarely performed, due to the lack of reagents, variability of the results and low sensitivity for the
meningo-encephalitic stage.\textsuperscript{39} Due to the problems with the sensitivity and specificity of the current disease stage parameters,\textsuperscript{40} alternative techniques for second stage diagnosis have been proposed such as detection of intrathecal IgM synthesis, IgM detection in CSF by a latex agglutination field test, detection of auto-antibodies against brain components and PCR.\textsuperscript{39;41-45} In imported cases, the increased white cell count and protein concentration often contribute to diagnosis of a neuro-inflammatory disease.\textsuperscript{2;3;12;18;46} The calculation of intrathecal synthesis after nephelometric analysis of albumin, IgG, IgA and IgM in paired serum and CSF, may represent a tool for further differential diagnosis.\textsuperscript{47} On the basis of disease-related CSF immunoglobulin patterns, a suggested diagnosis can be ruled out, or more specific analysis can be suggested.\textsuperscript{48} The intrathecal immunoglobulin pattern in sleeping sickness caused by \textit{T.b. gambiense}, is characterised by a two- to three-class immunoglobulin response with a dominant IgM synthesis in the CNS.\textsuperscript{39;42} A typical trypanosomiasis immunoglobulin pattern represented in quotient diagrams, is shown in figure 1. The intrathecal IgM fraction is always higher than the intrathecal IgG or IgA fraction and occurs in 98% of the patients with leukocyte counts higher than 20/µl. Blood-CSF barrier dysfunction in sleeping sickness is usually absent or mild, and occurs especially in very advanced late stage patients with strong IgM synthesis.

Of course the described pattern is not exclusive for trypanosomiasis. A similar pattern can occur in opportunistic infections of the brain with a three-class immune reaction, occasionally in neurosyphilis, in Lyme neuroborreliosis, in mumps meningo-encephalitis and in non-Hodgkin lymphoma involving the CNS.\textsuperscript{48} A dominant IgM class intrathecal response in combination with high immunoglobulin and especially, high IgM concentration in blood, should lead to strong suspicion of trypanosomiasis infection, which then should be confirmed by parasitological techniques. Unfortunately, often no immunoglobulin concentrations are determined in the cerebrospinal fluid.\textsuperscript{3;12;15} If the IgM concentration only is determined, the abnormally elevated IgM in CSF, which can be as high as 100 times the normal concentration,\textsuperscript{39} should draw the attention to African trypanosomiasis\textsuperscript{49}. The detection of oligoclonal bands\textsuperscript{12;18;46} has a low sensitivity for diagnosis of the meningo-encephalitic stage.\textsuperscript{50}

Conclusion
Clinicians outside Africa, considering a diagnosis of sleeping sickness in a patient with a history of travel or residence in endemic areas, are invited to contact the national reference centre for Tropical Medicine, CDC Atlanta or WHO Geneva for clinical consultation and provision of serodiagnostic and parasitological tests for facilitated diagnosis of this rare but lethal imported infection. Drugs to treat sleeping sickness are also made available through WHO and CDC.

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Conflict of interest

We have no conflict of interest to declare.
References


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Useful websites

http://www.who.int/health-topics/aftryp.htm

http://www.cdc.gov/ncidod/dpd/parasites/trypanosomiasis/default.htm

http://www.sleeping-sickness.com (in french)

http://www.who.int/tdr/publications/publications/aftryp_swg.htm

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Contact addresses

Drug supply

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CATT supply

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Search strategy and selection criteria

Data for this manuscript were identified by searches of Medline, references from relevant articles and through searches of the extensive files on trypanosomiasis and its diagnosis of the authors. Among the search terms were “sleeping sickness”, “Trypanosoma brucei gambiense”, “African trypanosomiasis”, “case report”, “diagnosis”, “treatment”. Case reports of more than 20 years old were omitted. Mainly English and French papers were reviewed, with exception of some case reports in German, Dutch or Spanish.
Figure 1. Typical quotient (CSF/serum concentration) diagrams for IgG, IgA and IgM with data from a *T. b. gambiense* patient in the meningo-encephalitic stage with 554 white cells/µl, trypanosomes in the CSF, and a CSF protein concentration of 711 mg/l. The patient’s CSF concentrations were 185 mg/l albumin, 234 mg/l IgG, 10.8 mg/l IgA and 160 mg/l IgM, blood concentrations were 25.3g/l albumin, 31.8 g/l IgG, 1.93 g/l IgA and 6.33 g/l IgM.

The reference ranges of blood derived IgG, IgA and IgM fractions in CSF are between the upper discrimination line (Q Lim, bold line) and lower discrimination line (dotted line). Q Lim represents the discrimination line between brain derived and blood derived immunoglobulin fractions in function of increasing Q Alb.48 Values indicated by dashed lines, above Q Lim, indicate intrathecal fractions (the % of the immunoglobulin concentration in the CSF of intrathecal origin). The plotted patient had a 3 class intrathecal immunoglobulin response with intrathecal fractions for IgG, IgA and IgM of respectively 26, 36, and 93%.

The age dependent vertical lines indicate the upper limit of the reference range for the age-related normal blood-CSF barrier function, at 15, 40 and 60 years of age. The patient had a moderate blood-CSF barrier dysfunction with Q Alb=7.31x10⁻³. Normal for his age of 30 would be 6x10⁻³.