

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

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Sleeping sickness is a lethal African disease caused by parasites of the *Trypanosoma brucei* subspecies, which is transmitted by tsetse flies. Occasionally, patients are reported outside Africa. Diagnosis of such imported cases can be problematic when the infection is due to *Trypanosoma brucei 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, neuro-pathological 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 should contact a national reference centre for tropical medicine in his or her country, or the WHO, Geneva, Switzerland, or the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, for clinical consultation and provision of specific diagnostic tests. Appropriate drugs for sleeping sickness treatment are also provided by WHO and the CDC.

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Sleeping sickness, also called human African trypanosomiasis, is caused by *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense* and is transmitted through the bites of tsetse flies.<sup>1–6</sup> 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 people in Africa is estimated at between 300 000 and 500 000. According to WHO (J Jannin, unpublished), about 20 *T b gambiense* and 30 *T b rhodesiense* infections are diagnosed yearly outside Africa, but migration, tourism, peacekeeping, and military interventions in areas at risk, in combination with the current sleeping sickness epidemic, might lead to increased numbers of cases outside Africa in the future. Unlike *T b rhodesiense* sleeping sickness, which is characterised by high parasite numbers in the blood and therefore

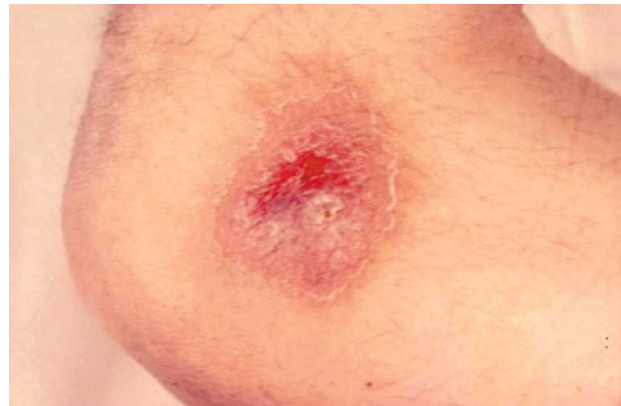


Figure 1. Trypanosomal chancre.

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is relatively easy to diagnose, imported *T b gambiense* sleeping sickness often remains unrecognised for years.<sup>7–14</sup> 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. By contrast, in endemic areas, active screening for sleeping sickness takes place by specialised mobile teams in some foci, and health workers are aware of the disease and know how to diagnose it.

Correct and rapid diagnosis of the disease is of utmost importance to 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 sequelae after treatment and possibly death of the patient.

## 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 (figure 1),<sup>15–17</sup> or much

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later in the disease course after development of serious neurological signs.<sup>7,9–11,13,14,18–21</sup>

Unlike in African natives, where the early stage of *T b gambiense* infection may pass almost without symptoms and remain unnoticed, the onset of trypanosomiasis is almost always acute in individuals from countries not endemic for the disease.<sup>20</sup> Whereas presence of a trypanosomal chancre has seldom been reported in Africans, it has been described at frequency of 25–40% in *T b gambiense* infected Europeans and may be the first sign of infection.<sup>20</sup> The chancre develops after 5–15 days at the site of the bite, expanding within a few days from an erythematous papule to a hard and painful nodule, several centimetres in diameter, with central ulceration. Within a few weeks, the chancre disappears without leaving a trace. In virtually all patients, a fever develops 1–3 weeks after the infecting tsetse bite. After the initial febrile attack, lasting for a maximum of 1 week, periods of fever become irregular, and may be separated by intervals of a few days to a month or more. The fever may lead to presumptive antimalarial treatment.<sup>12,16</sup> The trypanosomal rash, reported to occur in up to half of patients of European origin, is invisible on a dark skin. It presents as papuloerythematous eruptions affecting mainly the trunk and shoulders and is evanescent. Pruritus, often associated with skin rash, is seen 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 the 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 recognised—can be considered characteristic. Observation of one of these early symptoms should, in combination with a 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.<sup>22,23</sup> Neuroimaging data of trypanosomiasis are scarce.<sup>11,13,18,21,24</sup>

More detailed descriptions of the clinical presentation of *T b gambiense* sleeping sickness including imported cases are given in reviews by Duggan and Hutchinson,<sup>20</sup> Dumas and Bisser,<sup>22</sup> Edan,<sup>25</sup> and Boa et al,<sup>23</sup> and in 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,<sup>21</sup> and that the question of differential diagnosis may 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 raised IgM concentrations in blood should direct the diagnostic pathway towards more specific examinations. IgM concentrations in *T b gambiense* patients can be up to 16 times the normal concentration, as a result of polyclonal, non-specific B-cell activation. During the accompanying polyspecific immune response, a variety of non-trypanosome-specific antibodies and autoantibodies are produced—eg, against fibrin, fibrinogen, DNA, red blood cells, thymocyte antigens,<sup>26,27</sup> and central nervous system components such as myelin, galactocerebrosides, and neurofilament.<sup>28–30</sup> Antibodies specific for other pathogens, such as *Toxoplasma gondii*, *Strongyloides stercoralis*,<sup>18</sup> Epstein-Barr virus, cytomegalovirus,<sup>31</sup> *Plasmodium falciparum*, *Plasmodium brasiliana*, and *Borrelia burgdorferi*,<sup>8</sup> have been reported in patients with *T b gambiense* sleeping sickness and constitute an additional risk for misdiagnosis.

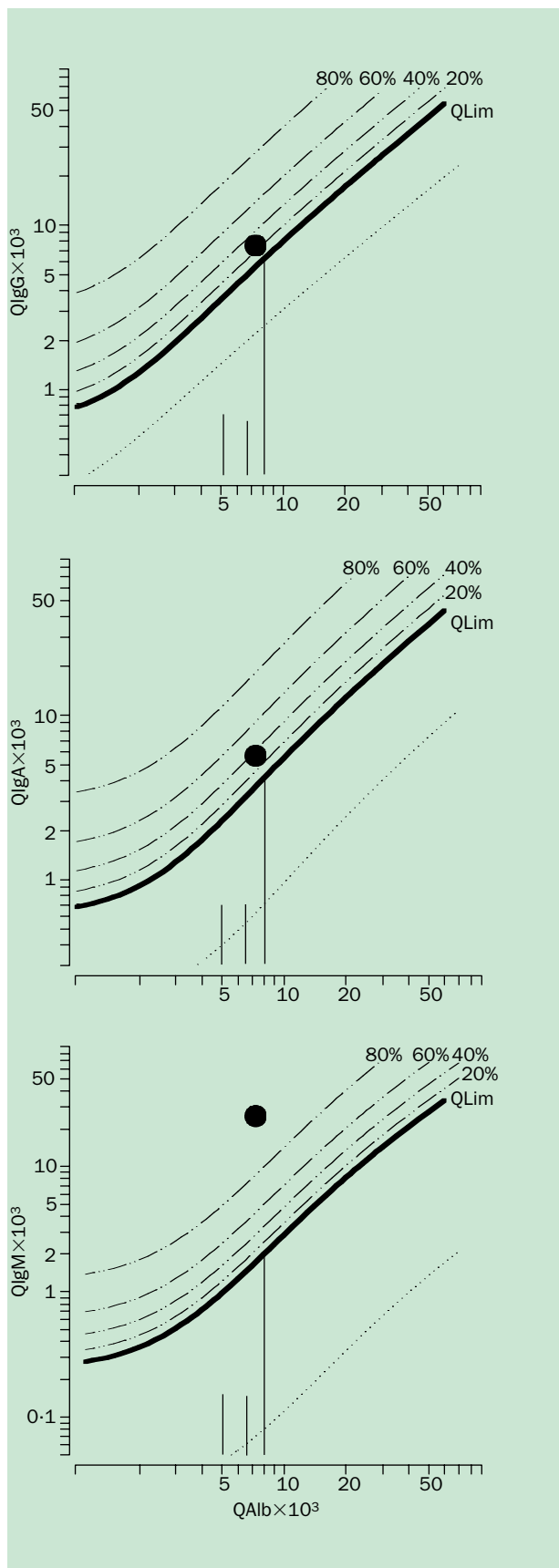
*T b gambiense* specific IgG and IgM antibodies are present in high concentrations and are mainly directed against the immunodominant surface glycoprotein antigens of the parasite. They can be detected by ELISA or immunofluorescence, with purified trypanosomal glycoproteins or whole trypanosomes of selected antigen types.<sup>32,33</sup> The test of choice when *T b gambiense* sleeping sickness is suspected is the card agglutination test for trypanosomiasis (CATT).<sup>34</sup> The CATT 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 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 the technique has insufficient sensitivity for detection of *T b gambiense* infections. A Giemsa-stained thick blood smear is more sensitive, but inexperienced laboratory technicians may have difficulty in recognising 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.

Specialised concentration techniques for trypanosome detection in blood can provide a more straightforward solution. The most sensitive technique, the mini-anion exchange centrifugation technique,<sup>35</sup> is not widely available. In non-specialised laboratories, application



of the microhaematocrit centrifugation technique<sup>36</sup> 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.

Experimental methods for trypanosome-specific nucleic acid detection by PCR have been described for the diagnosis of trypanosomiasis,<sup>14,37–40</sup> but PCR has not yet been fully validated. Problems with its reproducibility, and prolonged positivity after successful treatment have been reported.<sup>14,40</sup>

### Cerebrospinal fluid examination and stage determination

In sleeping sickness, cerebrospinal fluid (CSF) is usually examined for leucocyte count, total protein concentration, and the presence of trypanosomes. These examinations are done for discrimination between the haemolymphatic first disease stage and the meningoencephalitic second disease stage, which determines the choice of therapy. Pentamidine is used for treatment of haemolymphatic stage *T b gambiense* trypanosomiasis and is relatively well tolerated. However, it is inefficient in the meningoencephalitic 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 that occurs in 5–10% of treated patients and is fatal in half of them.<sup>41,42</sup>

Second-stage trypanosomiasis is diagnosed in patients with a CSF leucocyte count of more than 5 cells/ $\mu$ L or with trypanosomes in CSF. The latter can be detected in the cell-counting chamber, or by double or simple centrifugation of the CSF.<sup>43,44</sup> In Africa, total protein determination in CSF is only rarely done, due to lack of reagents, variability of the results, and low sensitivity for the meningoencephalitic stage.<sup>45</sup> Due to the problems with the sensitivity and specificity of the current disease-stage parameters,<sup>46</sup> alternative techniques for second-stage diagnosis have been proposed such as detection of intrathecal IgM synthesis, IgM

Figure 2. Typical quotient (CSF/serum concentration) diagrams for IgG, IgA, and IgM<sup>64</sup> with data from a *T b gambiense* patient in the meningoencephalitic stage with 554 white cells/ $\mu$ L, 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 25.3 g/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 as a function of increasing  $Q_{Alb}$ .<sup>54</sup> 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 three-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  $Q_{Alb}$ =7.31 $\times$ 10<sup>3</sup>. Normal for this patient aged 30 would be 6 $\times$ 10<sup>3</sup>.

## Contact addresses

## Drug supply

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## Useful websites

<http://www.who.int/health-topics/afrtryps.htm> (accessed Oct 28, 2003)

[http://www.who.int/tdr/publications/publications/afrtryp\\_swg.htm](http://www.who.int/tdr/publications/publications/afrtryp_swg.htm) (accessed Oct 28, 2003)

<http://www.cdc.gov/ncidod/dpd/parasites/trypanosomiasis/default.htm> (accessed Oct 28, 2003)

<http://www.sleeping-sickness.com> (in French; accessed Oct 28, 2003)

detection in CSF by a latex agglutination field test, detection of autoantibodies against brain components, and PCR.<sup>45,47–51</sup>

In imported cases, the increased white cell count and protein concentration often contribute to diagnosis of a neuroinflammatory disease.<sup>8,9,18,24,52</sup> 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.<sup>53</sup> On the basis of disease-related CSF immunoglobulin patterns, a suggested diagnosis can be ruled out, or more specific analysis can be suggested.<sup>54</sup> The intrathecal immunoglobulin pattern in sleeping sickness caused by *T b gambiense*, is characterised by a two to three-class immunoglobulin response with dominant IgM synthesis in the central nervous system.<sup>45,48</sup> A typical trypanosomiasis immunoglobulin pattern represented in quotient diagrams is shown in figure 2. The intrathecal IgM fraction is always higher than the intrathecal IgG or IgA fraction and occurs in 98% of the patients with leucocyte 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

## 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”, and “treatment”. Case reports from more than 20 years ago were omitted. Mainly English and French papers were reviewed, with exception of some case reports in German, Dutch, or Spanish.

immune reaction, occasionally in neurosyphilis, in Lyme neuroborreliosis, in mumps meningoencephalitis, and in non-Hodgkin lymphoma involving the central nervous system.<sup>54</sup> 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 CSF.<sup>9,18,21</sup> If the IgM concentration only is determined, abnormally raised IgM in CSF, which can be as high as 100 times the normal concentration,<sup>45</sup> should draw attention to African trypanosomiasis.<sup>55</sup> Detection of oligoclonal bands<sup>18,24,52</sup> has a low sensitivity for diagnosis of the meningoencephalitic stage.<sup>56</sup>

## 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 a national reference centre for tropical medicine in their country, or WHO, Geneva, Switzerland, or the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, for clinical consultation and provision of serological and parasitological tests for diagnosis of this rare but lethal imported infection. Drugs to treat sleeping sickness are also made available through WHO and the CDC (panel).

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## Conflicts of interest

We have no conflict of interest.

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