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## REVISED MANUSCRIPT

# The Challenging Problem of Disease Staging in Human African Trypanosomiasis (Sleeping Sickness): a new approach to a circular question

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## **Abstract**

Human African trypanosomiasis (HAT), also known as sleeping sickness, puts millions of people at risk in sub-Saharan Africa and is a neglected parasitic disease that is almost always fatal if untreated or inadequately treated. HAT manifests itself in two stages that are difficult to distinguish clinically.

The problem of staging in HAT is extremely important since treatment options, some of which are highly toxic, are directly linked to the disease stage. Several suggested investigations for disease staging have been problematic because of the lack of an existing gold standard with which to compare new clinical staging markers. The somewhat arbitrary current criteria based on the cerebrospinal fluid (CSF) white blood cell (WBC) count have been widely used, but the new potential biomarkers are generally compared with these, thereby making the problem somewhat circular in nature.

We propose an alternative 'reverse' approach to address this problem, conceptualised as using appropriate statistical methods to test the performance of combinations of established laboratory variables as staging biomarkers to correlate with the CSF WBC/trypanosomes and clinical features of HAT. This approach could lead to the use of established laboratory staging markers, potentially leading to a gold standard for staging and clinical follow-up of HAT.

## Introduction

Human African trypanosomiasis (HAT), commonly known as sleeping sickness, is caused by an infection with protozoan parasites belonging to the genus *Trypanosoma* and puts 60 million people at risk throughout 37 countries in sub-Saharan Africa.<sup>1,2,3</sup> The trypanosome parasites are transmitted to humans by the bite of tsetse fly (of the *Glossina* genus) which has acquired the infection from human beings or from animals harbouring the human pathogenic parasites.<sup>4,5</sup> Almost always fatal if untreated or inadequately treated, HAT is an extremely important but also 'neglected disease' in Africa with neglected associated stigma.<sup>6</sup> It expresses itself clinically in two distinct forms in two broadly separate regions of Africa.<sup>7</sup> The parasite *Trypanosoma brucei gambiense* (*T.b. gambiense*) is the causal agent of the chronic West African disease variant form which is endemic in 24 countries in Central and West Africa, accounting for about 97% of reported cases, while *Trypanosoma brucei rhodesiense* (*T.b. rhodesiense*) is responsible for the more acute East African variant, endemic in 13 countries in East and Southern Africa and accounting for about 3% of reported cases.<sup>3</sup> However, it has recently been shown that *T.b. rhodesiense* represents 18% of the total risk from HAT.<sup>8</sup> Both these variants of HAT are known to coexist in Uganda raising the very realistic possibility that in the future some patients in this region may be dually infected with both *T.b. rhodesiense* and *T.b. gambiense*,<sup>9,10</sup> even though some attempts have been made at controlling the threat.<sup>11,12</sup>

The disease can be conceptualized as a complex entity, the full nature of which cannot be fully appreciated when observed from just a single perspective. This situation could be improved, were it possible to observe the disease in its entirety to facilitate a better understanding of the full situation. The curious duality characterizing HAT is not limited to aetiological and geographical duality but extends to a duality of both the immune response and disease progression,<sup>13,14</sup> from stage 1, also known as the haemolymphatic stage to stage 2, also known as the encephalitic stage when the parasites have traversed the blood-brain barrier (BBB) to invade the central nervous system (CNS).<sup>15</sup> Although much has been achieved through research over the years in the characterization of and distinction between the two aetiological agents and the different geographical disease forms, less progress has been made in the understanding and definition of the two stages of HAT. The issue of disease staging is crucial for treatment of the CNS form of the disease and follow-up in HAT, but the insufficiency of objective criteria for staging is a significant challenge to clinicians treating sufferers of this disease. According to the WHO 2012 technical report on research priorities for three major including HAT,<sup>16</sup> "the development of a better and more user-friendly marker for staging was identified by a TDR expert group as number one priority in HAT diagnostics. The need for lumbar puncture is indeed one of the most important constraints to the uptake of HAT screening programmes."

## The Problem of Disease Staging in HAT

HAT progresses from an early, stage 1 or the hemolymphatic stage to the late, stage 2 or encephalitic stage in an acute or chronic fashion in the *T.b. rhodesiense* or *T.b. gambiense* form of the disease respectively. In the early stage, the trypanosomes multiply in the blood, lymphatic system and systemic organs giving rise to a variety of symptoms and signs some of which may be quite non-specific.<sup>7</sup> In the later more severe stage, the parasites cross the BBB and migrate to brain parenchyma to cause a wide range of neurological features the hallmark of which is the disturbance of sleep structure and sleep-wake rhythm.<sup>7</sup> In the human disease it is not known precisely when this traversal through the BBB is initiated or completed, and there is still a need to better characterize the timing and evolution of the late stage signs and symptoms in order to construct an objective clinical score that could be a useful adjunct to staging. There are unfortunately no stage-specific clinical features that can reliably distinguish the early from the late stages of HAT where in both disease forms the stages merge into each other. The question of staging in trypanosomiasis has been more easily and mechanistically addressed in experimental models of the disease. For example, late stage disease corresponding to the crossing of the BBB by the parasite has been shown to start to occur between days 11 and 13 after infection.<sup>17</sup> Furthermore, the mechanism of crossing the BBB in this model has been investigated in detail.<sup>18</sup> However, the timing of these events in the human disease, let alone the molecular mechanisms involved are far more challenging questions to address than they are in animal models where the conditions can be experimentally manipulated and controlled. The situation is somewhat complicated by recent evidence in mouse models of HAT that trypanosomes may enter the brain parenchyma,<sup>19, 20</sup> and meninges,<sup>21</sup> at much earlier times than had been previously thought. Such experimental findings may also have relevance to recent observations in Uganda that patients with *T.b. rhodesiense* may show neurological symptoms and signs during the first stage, as well as the proven second stage, of the disease.<sup>15</sup>

In either the acute or the chronic form of the disease, the determination of the stage is the critical determinant of the treatment options for both variants of HAT. The drugs used for early stage disease (suramin for *T.b. rhodesiense* and pentamidine *T.b. gambiense*) are not used or if used, are ineffective in the late stage of the disease. Drug treatment for late stage disease is highly toxic, making accurate disease staging a critically important issue. The only currently available treatment for late stage *T.b. rhodesiense* infection (has been used also for *T.b. gambiense*) is intravenous melarsoprol which, while usually effective, results in a severe post-treatment reactive encephalopathy in about 10% of cases of whom at least half die as per several reviews.<sup>22, 23</sup> In the more recent 10-day melarsoprol regime the drug was associated with an overall mortality of 5.9% in *T.b. gambiense*,<sup>24</sup> and ~8% in *T.b. rhodesiense* disease.<sup>25</sup> The

new primary treatment for late stage *T.b. gambiense* disease is nifurtimox-eflornithine combination therapy (NECT) which has a lower toxicity and mortality than melarsoprol but was still associated with an overall mortality of 0.7% in a key clinical trial.<sup>26</sup> Moreover, intravenous melarsoprol still remains the second line therapy for late stage *T.b. gambiense*. Given that to date, clinical symptoms and signs have not been sufficient for staging,<sup>15</sup> current staging is based on biological data, essentially on cerebrospinal fluid (CSF) analysis. However, it is appreciated that if safe oral therapies for CNS HAT do become widely available then this issue of diagnostic staging will inevitably become less urgent than it is at present. Indeed such a new therapy could potentially obviate the pressing need to distinguish early from late-stage disease.

The most commonly used CSF criteria used in the field for HAT staging are those defined by the World Health Organization (WHO) criteria.<sup>27</sup> These criteria for HAT stages are as follows:

- early stage: absence of trypanosomes in CSF, equal or less than 5 WBC/mm<sup>3</sup> in CSF,
- late stage disease: presence of trypanosomes in CSF and/or more than 5 WBC/mm<sup>3</sup> in CSF.

These criteria pose several difficulties:

1. Although highly specific and relatively easy to perform, the detection of trypanosomes in the CSF is not very sensitive,<sup>28</sup> as parasites are not always found in late stage patients (unpublished data);
2. The complementary criterion of number of WBC may be somewhat arbitrary as some national HAT control programmes use different cut-offs (up to 20 WBC/mm<sup>3</sup>) for treatment;<sup>19</sup> thus there is no universal consensus on the optimum CSF criteria for staging.
3. Reports exist in the literature of patients with WBC between 5 and 20/mm<sup>3</sup> but having no parasites, or patients with parasites but equal or less than 5 WBC/mm<sup>3</sup> that have been successfully treated and cured with early stage drugs.<sup>28</sup> In general, we would regard the presence of trypanosomes in the CSF as unequivocal evidence of CNS invasion.
4. All the above demonstrates the absence of a generally accepted 'gold standard' for disease staging in HAT.<sup>4, 19</sup> The clinical implications of the prevailing situation are serious.
5. Both false negative and false positive results may therefore be obtained in principle. In the former case the patient will die from lack of appropriate treatment, and in the latter case a patient with early stage disease will be exposed unnecessarily to the risk of highly toxic drugs normally given for CNS stage disease.<sup>4</sup>

In addition to these difficulties, the literature suggests that the spectrum of HAT stages spans possibly from the asymptomatic form through the early, early-late, intermediate and finally late stages.<sup>7,29,30,31</sup> A further difficult issue is that of 'relapses' which could occur in any of the stages, from early to late, if the infection is not sufficiently treated for several reasons. Can the asymptomatic cases constitute a stage of their own? This relatively new concept of the asymptomatic form of HAT,<sup>29, 30, 31</sup> which is closely related to the underlying process of animal trypanotolerance and the incompletely understood interplay between parasite and host mechanisms, is a very interesting one and offers a potentially ethically acceptable scenario where the natural history of the disease can be observed in the field. Further, there may exist an 'intermediate stage' of infection in which a small number of WBC are detected in the CSF but where parasites have crossed the BBB but not yet spread to the brain parenchyma,<sup>32, 33</sup> and perhaps these patients could potentially be candidates for treatment with early stage drugs. This remains a controversial concept, and we would not advocate at present the use of suramin or pentamidine for patients with a CSF WBC of >5/mm<sup>3</sup>.

There have been several reports of new biological CSF biomarkers or combinations of biomarkers, which have been suggested as potential staging tools for HAT.<sup>28, 34,35, 36, 37, 38, 39, 40</sup> While certainly of considerable interest both biologically and clinically, they all suffer from the same drawback which is that they are compared in sensitivity and specificity with the WHO CSF criteria as well as in some cases neurological features,<sup>36</sup> which, as we have seen, are themselves considered to be problematic in the first place. This approach, while entirely understandable, constitutes a form of inevitable "circular argument" because of the lack of a gold standard for comparison. This continues to be a major issue in this area both for the clinicians and researchers. Some authors have reported that early stage patients who demonstrated detectable levels of intrathecally synthesized Ig did not relapse after 12 months of follow-up and this in both chronic and acute forms of the disease.<sup>15, 41</sup> This suggests that intrathecal Ig synthesis, though not a specific or sensitive parameter for identification of late stage disease, may be interesting as a potential marker of early stage HAT. It has also been shown that a combination of CSF CXCL10, lipocalin 2 and secretory leucocyte peptidase inhibitor (SLPI) protein levels correlated with late stage HAT (as defined by the WHO criteria) compared with early stage disease in patients from Malawi and the Democratic Republic of Congo.<sup>42</sup> A recent paper has highlighted the potential role of neopterin as a promising biomarker for staging *T.b. gambiense* HAT patients,<sup>28</sup> and proposed the development of a rapid test for detecting neopterin in CSF for more accurate disease staging. Neopterin was found to be the best discriminator between patients with and without CSF parasites, and neopterin also predicted the presence of neurological signs with the same ability as both IgM levels and the CSF WBC.<sup>28</sup> This is clearly an encouraging development which needs to be studied further, but it is important to bear in mind that (a) the patients studied were classified as being in stage 2 based on the WHO CSF criteria and (b) as already noted neurological features may also occur in early stage disease. In the context of this cyclic problem of looking for staging tools for HAT without

any gold standard, it perhaps makes sense to also focus research efforts on the asymptomatic cases, the early stage patients who relapse, and the so-called intermediate stage patients.

The general inadequacy of the above biomolecular criteria to provide a consensus for staging methods for HAT has led to the evaluation of non-invasive biomarkers and this has included electrophysiological parameters, with the goal of finding surrogate markers to complement current staging tools. The description and use of polysomnography, in particular Sleep Onset Rapid Eye Movement periods (SOREMPs) by Buguet and colleagues,<sup>14</sup> raised the hope for this latter electrophysiological trait to become a biomarker of late stage HAT. However, SOREMPs have been demonstrated to be present both in early and late stages of the disease in the experimental and as well as the human disease,<sup>17,43</sup> and in the animal model, the occurrence of SOREMPs has been demonstrated to precede the crossing of the BBB by trypanosomes.<sup>17</sup> SOREMPs could, however, constitute a useful non-invasive tool for detecting disease relapses.

Despite the place of polysomnography and SOREMPs as the gold standard in sleep medicine, this technology poses several practical challenges in field conditions and is relatively expensive and largely unavailable in most African countries where HAT is endemic. In view of this, we have recently carried out a pilot study to show that a simple, relatively cheap and user-friendly field adaptable technology called actigraphy could be promising as a complementary tool for more accurate non-invasive staging of HAT.<sup>43</sup> The technique involves the use of a small instrument like a wrist watch known as an actigraph, that measures body movements (that are used to derive sleep-wake patterns), alterations of which compared to normal individuals or those in early stage disease appears to correlate with late stage HAT.<sup>43</sup> The electrophysiological hallmark of HAT is the total loss of the sleep structure with nocturnal insomnia and diurnal hypersomnolence even in stage 1 patients and comparison with simultaneous polysomnography. Furthermore, we showed that the polysomnographic and actigraphic alterations in HAT patients did not correlate with the CSF WBC count, demonstrating the need to find a new approach to solving the HAT staging problem. Actigraphic techniques are promising but require much further investigation and also validation against the WHO CSF staging criteria particularly the unequivocal presence of trypanosomes for stage-2 disease.

It should be appreciated that the important issue of HAT disease staging is one of several key aspects of HAT management and control that need to be addressed at the same time. These other important aspects that also have high priority include i) the detection of asymptomatic patients, (ii) the improvement of serological and molecular diagnostic methods, (iii) the assessment of new drugs, (iv) the access to and improvement of diagnostic facilities, and (v) the implementation of integrated strategies combining vector control with detection and therapy of HAT cases.

## **A Potential Novel Approach to the HAT staging problem**

We suggest that there may be another potential approach to HAT staging which can be conceptualized as a kind of 'reverse' methodology. Rather than attempting to correlate a new diagnostic staging tool with the current WHO CSF WBC staging criteria, we suggest that another approach would be to select say four known biomarkers of CNS disease activity and then determine from field studies how well these correlate, alone or in various combinations, with different CSF WBC and more importantly with the presence or absence of trypanosomes in the CSF.

For example, one could take neopterin, IgM, CXCL10 and IL-10 as biomarkers and then determine the extent to which individual levels of these in the CSF of early and late stage patients differ from baseline values. These individual values and in combination would then be explored for correlation with observed CSF WBC levels and CSF presence of trypanosomes using statistical multiple variable regression methods. For a multiple regression predictive model, the biomarker values would be the explanatory variables and typically provide a linear relationship with the dependent variable CSF WBC. The presence of CSF trypanosomes, however, would always remain a defining variable for CNS stage disease. Statistical evaluations would determine the biomarkers which best predict CSF WBC levels and exclude those with no predictive power.

Biomarkers and CSF WBC values are measured in different units but can be standardised as are the presence or quantification of trypanosomes, before undertaking regression by subtracting their mean and dividing by their standard deviation so that they are measured from their means in units of standard deviations. Multiple regression analysis applied to such data then provides standard partial regression coefficients for each of the biomarkers. A biomarker which has a standardised regression coefficient twice as large as the coefficient of another biomarker is likely to be twice as important in predicting CSF WBC level.

Alternatively CSF WBC and each of the biomarkers can be categorised as high or low based on current medical knowledge. The response variable and the explanatory variables are then no longer continuous but dichotomous. This leads to the adoption of logistic regression which uses the same multiple regression methods to predict the probability of a high CSF WBC value using the high and low biomarker classifications. More important the regression coefficients (when raised to the power of the exponential constant) for each of the biomarkers provide estimates of odds ratios. The odds ratios measure the increase in odds of high CSF WBC when a high biomarker value is obtained. However, caution must be exercised with these four suggested biomarkers as they may also be raised in other infectious diseases and by themselves are not necessarily specific to HAT.

There are other possibilities for multiple regression methods according to what data could be made available.<sup>44</sup> For example if data were available according to early or late stage on cerebrospinal fluid (CSF), polysomnography (PSG), actigraphy (which are more specific) etc.

then statistical modelling could be used to explore the potential of a wide range of explanatory variables. Analysis of empirical data for HAT staging using multiple regression methods offers a way forward. Such data are not easily available and looking at multiple variables increases complexity and cost. Typically multiple regression studies require circa 100 subjects for estimating multiple correlation relationships and testing individual predictors, with the detail depending on statistical power, significance and effect sizes, but nevertheless valuable knowledge is to be gleaned from studies with fewer patients.

### **Concluding remarks**

Human African trypanosomiasis (HAT), or sleeping sickness, is a major killer disease in sub-Saharan Africa but the treatment of late stage disease when the CNS is invaded by the trypanosome parasites remains problematic due to severe drug toxicity. Accurate staging of HAT is therefore crucial to identify optimum therapy. There is not a universal consensus about the current WHO criteria for CNS HAT, and there is a tendency for potential new CSF diagnostic markers to be compared with the validity of the existing WHO criteria which is a circular argument. In this analysis we suggest a novel way of addressing this diagnostic staging problem by employing statistical analyses to test the efficacy of different combinations of existing field-validated biomarkers to correlate with the CSF WBC/trypanosomes and also clinical features of HAT.

## Author contributions

All three authors contributed equally to the writing of this review

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