Difficulties in Diagnostic Staging of Human African Trypanosomiasis

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Abstract Human African trypanosomiasis (HAT), also known as sleeping sickness, continues to be a major hazard to human health in 36 countries in sub-Saharan Africa. One of the most important problems in disease management is the considerable difficulty in distinguishing the early (hemolymphatic) from the late (encephalitic) stage when the parasites have crossed the blood-brain barrier to enter the Central Nervous System (CNS). Treatment of the two stages is different with the highly toxic arsenical drug melarsoprol being the most commonly used therapy for CNS disease. Since melarsoprol kills 5% of treated patients, it is vital to develop reliable and agreed diagnostic staging markers for HAT. Although the current WHO staging criteria on the cerebrospinal fluid (CSF) are the most commonly used, there is still a lack of consensus as to their efficacy which has driven the current search for improved methods of HAT diagnostic staging which are considered here.

Keywords human African trypanosomiasis; sleeping sickness; central nervous system (CNS); cerebrospinal fluid (CSF); diagnostic staging

1 Introduction

Human African trypanosomiasis (HAT), or sleeping sickness, remains as one of the most problematic of Africa’s neglected diseases, causing major disruption of human health and economic productivity throughout 36 countries in sub-Saharan Africa where 60 million people are at risk of contracting the disease [7]. Transmitted by the bite of the tsetse fly, HAT is caused by protozoan parasites of the genus Trypanosoma [7]. The two forms of the disease are Trypanosoma brucei gambiense (T.b.gambiense) which causes West African HAT, and T.b.rhodesiense which causes the much less prevalent East African HAT. The former variant has a slower and more protracted disease course compared with rhodesiense disease which can kill patients in weeks to a few months rather than a year or more.

During the early stage of HAT, also known as stage 1, or the hemolymphatic stage, the parasites invade and spread within the blood, lymphatic system and systemic organs. After about 3 weeks, the disease progresses to the late stage, also known as stage 2, or the encephalitic stage, where the parasites cross the blood-brain barrier (BBB) to enter the central nervous system (CNS) [7,9]. Once the CNS is invaded the patient may suffer from a constellation of neurological symptoms and signs including motor, psychiatric and sensory disorders and disruption of normal sleep/wake cycles [9]. If untreated or inadequately treated, all patients with HAT will eventually die. Despite this, the drug treatment of HAT is itself highly problematic as all the drugs in current use are unavailable orally and toxic. The most commonly used late stage drug for treating both forms of the disease, the arsenical melarsoprol, produces a severe post-treatment reactive encephalopathy (PTRE) in 10% of patients, half of whom die [7,9]. This remarkable overall 5% fatality rate of melarsoprol underpins the critical requirement for accurate diagnostic staging markers for distinguishing the early and late stages of HAT, since the early stage drugs (suramin and pentamidine), while certainly not free of side-effects, do not have this very high degree of lethal toxicity. While melarsoprol is the only available treatment for rhodesiense HAT, an alternative regime for gambiense disease has recently been instigated with eflornithine and nifurtimox [14], but these drugs are also toxic, though less so than melarsoprol [9]. Unfortunately, current diagnostic staging for HAT remains problematic and needs to be improved urgently.

Currently, a lumbar puncture, an invasive procedure, is carried out to examine the cerebrospinal fluid (CSF) in HAT patients to distinguish the early from the late stage of infection. The WHO criteria for CNS-stage disease are: the presence of trypanosomes and/or more than 5 white blood cells (WBC)/μL in the cerebrospinal fluid (CSF) [3]. The presence of trypanosomes is diagnostic but they are not usually detectable. Because these WBC levels may lead to potentially false positive results, not all experts follow these guidelines and some have adopted alternative criteria, ranging from 6 WBC/μL–20 WBC/μL [8,10]. This represents a major management problem since failure
to diagnose and treat CNS HAT will result in inevitable death, but treating patients who do not actually have CNS disease exposes them to the unnecessary risks of toxic drugs such as melarsoprol [9]. This is one of the great diagnostic dilemmas in treating HAT patients. Also, the criteria for defining the existence of late-stage disease are not necessarily the same as those adopted as the basis for toxic late-stage drug therapy [7,8]. It is also possible that there may be an “intermediate stage” of infection with a CSF WBC of < 20/μL where the trypanosomes have penetrated the BBB but not extensively invaded the brain, but this remains a controversial notion and caution should be exercised in suggesting that such patients should or could be treated with early-stage drugs [7,8].

Because of the lack of a general consensus, the current WHO CSF WBC criteria for identifying late stage HAT cannot be regarded as a “gold standard”. However, since these are the most widely used and recognized criteria, any new potential staging method will inevitably be compared with them. This represents a form of “circular argument” but it is difficult to circumvent this problem in the absence of an alternative definitive diagnosis of CNS involvement. In the future, advances in diagnostic imaging allow eventually new methods of visualizing parasites traversing the BBB and established within the CNS to enhance diagnostic certainty. Nevertheless, there have been a number of recent attempts to devise alternative or adjunctive CSF diagnostic staging criteria using a range of novel biomarkers, often in combination, and sometimes tested in both animal models and patients in the African field.

A promising recent approach has been to use a panel of biomarkers to identify late-stage HAT reliably. In animal models, the latter can be defined practically as the stage during experimental infection at which early stage drugs such as suramin and pentamidine are no longer effective at producing a cure [11]. In patients, the biomarkers can be correlated with CSF findings, usually the WHO criteria that define late-stage disease. Hainard et al. [5] reported the use of the chemokines CXCL10, CXCL8 and the heart-fatty acid binding protein (H-FABP) to distinguish the early and late stages of HAT with a high degree of both specificity and sensitivity in gambiense patients. In a somewhat similar approach, Amin et al. [1] found that in a mouse model of HAT a microarray analysis showed increased expression of transcripts for lipocalin 2 and secretory leukocyte peptidase inhibitor (SLPI) at the late stage of infection. Furthermore, when these latter markers were examined in patients’ CSF in the Democratic Republic of Congo (DRC) (90 cases of both early and late stage disease), it was found that levels of lipocalin 2, SPLI and CXCL10 protein were all significantly increased in late stage disease. Interestingly, these elevations were not correlated with either the presence of somnolence, CSF trypanosome numbers or intrathecal IgM titers [1]. The latter is also significant because it has been clearly shown that the CSF IgM level is a very useful marker of CNS disease in HAT under field conditions [12]. Thus, the various potential biomarkers do not necessarily correlate with one another.

Recently, two new candidate biomarkers have been described by Tiberti et al. in HAT patients with T.b.gambiense in the DRC [15]. Using initial two-dimensional gel electrophoresis subsequently confirmed by Western blot and ELISA, these authors found that the two proteins osteopontin and β-2 microglobulin were good potential biomarkers of late-stage disease, with promising sensitivities and specificities when correlated with the WHO CSF staging criteria. Very recently, it was shown that the performance of another well known technique, the polymerase chain reaction (PCR), was equal to or better than the current parasite detection techniques for T.b. gambiense diagnosis and staging, but it could not be used for post-treatment follow-up [4]. Another recently published study by Hainard et al. [6] reported that the intercellular adhesion molecule 1 (ICAM-1) and matrix metalloproteinase-9 (MMP-9), either alone or in combination, were powerful CSF staging markers for HAT patients with gambiense disease. Again, the performance of these markers was established in relation to their correlation with CSF parasite detection and/or the number of WBC.

What is to be made of these various promising reports? Clearly, it is very encouraging to have available novel biomarkers for staging diagnostics, especially when they are more biologically meaningful than a CSF pleocytosis alone. While their potential efficacy has been well demonstrated in the various studies, there still remain the two difficult issues of the true validity, both biologically and clinically, of the WHO CSF staging criteria themselves, and the practical challenges of carrying out these sophisticated analyses widely under African field conditions. Whether any new diagnostic test will be of primary diagnostic value, or else adjunctive to the WHO criteria, remains to be seen. Ideally, any new diagnostic staging test for HAT needs to be cheap, quick and easy to perform, and easily adapted to field conditions [10].

There are two potential developments that would obviate significantly many of the current difficulties in diagnostic staging in HAT. First, the establishment of a highly sensitive and specific non-invasive method would be of great benefit. Currently, polysomnography is a well-established non-invasive procedure that has an identified characteristic sleep onset of rapid eye movements as indicating an alteration of sleep structure that correlates with the onset of late-stage disease [2,13]. Whether this technique can eventually be used as either a primary or adjunctive diagnostic staging tool for HAT is likely to depend on the extent to which it can be closely and rigorously correlated with the characteristic
CSF abnormalities seen in late-stage disease. This technique already has a considerable potential for detecting relapses of CNS HAT. Potentially, there may be other non-invasive techniques that can also be developed for diagnostic staging. A second major advance in the future would be the development of a safe oral therapy for CNS HAT, as opposed to the very toxic drugs in current use. Such an advance would be a major one as it would obviate the very difficult problems that we still face when attempting to distinguish reliably between early and late stages of HAT.

References


