Editorial

Novel Biomarkers for Late-Stage Human African Trypanosomiasis—The Search Goes On

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Human African trypanosomiasis (HAT), or sleeping sickness, is one of the most problematic of the parasitic diseases of sub-Saharan Africa, where 60 million people are at risk from the infection. Caused by protozoan parasites of the genus *Trypanosoma* and transmitted by the bite of the tsetse fly, the disease has two forms: East African HAT caused by *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) and West African HAT caused by *T. b. gambiense*.¹ During the early or hemolymphatic stage, the parasites spread within and are confined to the blood, lymphatic system, and systemic organs. In the late or encephalitic stage, the trypanosomes cross the blood–brain barrier (BBB) and invade the central nervous system (CNS) to cause a constellation of neurological disorders including the characteristic sleep disturbance that gives the disease its name.² If HAT is untreated or inadequately treated, all patients will eventually die. But current drug treatment of HAT, especially in late stage, is highly toxic. Melarsoprol, unlike early-stage drugs such as suramin and pentamidine, crosses the BBB and is the most commonly used drug for both types of CNS HAT, but it is associated with an overall fatality rate of 5% because of a severe reactive encephalopathy.³ The ability to stage accurately both early and late HAT is, therefore, vital.

It is in this staging context that the findings of Amin and others⁴ in both mice and humans in the current issue of the *American Journal of Tropical Medicine and Hygiene* are of particular interest. They point out that current biomarkers of CNS HAT are problematic. The most commonly used criteria for late-stage disease were devised by the World Health Organization (WHO) and include the presence of trypanosomes in the cerebrospinal fluid (CSF) and/or a CSF white blood cell (WBC) count of $> 5/\mu\text{L}$.⁵ But not everyone agrees with these criteria, and in some areas, patients presenting with up to 20 WBC/ μL have been treated successfully with the early-stage drug pentamidine, although this approach remains controversial.⁶ Therefore, by applying current parameters, both false-positive and false-negative results are possible with serious consequences for patients.⁷ Thus, there is no absolute consensus on either the definition of CNS disease or the criteria for its treatment.¹ Amin and others⁴ adopted a different approach and used a mouse model of HAT to investigate if molecules secreted from the brain parenchymal cells, rather than molecules secreted by WBC, could act as biomarkers for late-stage disease.

Amin and others⁴ defined late-stage disease in mice as a failure to be cured by the early-stage drug suramin, and they used microarray technology to detect transcripts differentially

expressed in these brains after CNS trypanosome infection. It was found that transcripts for lipocalin 2 and secretory leukocyte peptidase inhibitor (SLPI) were the most highly expressed in the brains of mice at a late compared with an early stage of *T. b. brucei* infection. They also detected differential up-regulation on microarrays of lipocalin 2, SLPI, and the chemokine CXCL10 in the brains of rats at the late stage of trypanosome infection; this also showed significantly increased levels of CSF lipocalin 2. Their study was strengthened by the inclusion of HAT patients from the Democratic Republic of Congo and Malawi in these analyses. When equal numbers (90) of early- and late-stage HAT patients were compared, it was found that the CSF protein levels of lipocalin 2, SPLI, and CXCL10 were significantly elevated in late-stage (based on the WHO CSF criteria) compared with early-stage patients. Interestingly, no correlation was detected between lipocalin 2, SPLI levels, and clinical features, such as somnolence or laboratory parameters like the number of trypanosomes in the CSF or intrathecal IgM titers.

Caution should always be used in extrapolating results obtained in animal models to the human scenario, but the elevations of these three molecules in the CSF of HAT patients clearly indicate the diagnostic potential of these findings. The late-stage rise of CNS CXCL10 in rats and the CSF of HAT patients confirms the earlier study by Amin and others,⁸ and it was also recently reported by Hainard and others,⁹ who showed that a combined panel of CXCL10, CXCL8, and heart-fatty acid binding protein (H-FABP) could be used to distinguish the early and late stages of *T. b. gambiense* HAT with very high sensitivity and specificity.⁹ Both these studies used the WHO criteria for defining late-stage disease. Although this is clearly essential at present, any new diagnostic assay should ideally be compared with a gold standard, which is somewhat difficult to achieve given the current lack of universal consensus regarding the WHO criteria. This underlies an inherent, somewhat circular, problem with devising better diagnostic markers for HAT. It is notable that, in the study by Amin and others,⁴ not only were the elevated CSF proteins not correlated with the patients' clinical or laboratory parameters, but there were also several of patients classified as having late-stage disease based on the WHO criteria who showed no increases in levels of lipocalin 2, SLPI, or CXCL10. If these molecules are to be useful as staging biomarkers, it will be very important to study larger numbers of patients and to retain an awareness of the most appropriate clinical and laboratory parameters with which to correlate any changes.

The WHO criteria for diagnosing late-stage HAT will continue to be used widely, but there is a powerful case for developing improved biomarkers for late-stage disease. Although a non-invasive test would be ideal, disease staging, in reality, will continue to rely on CSF analysis for the foreseeable future. The chemokine CXCL10 is emerging as a clear favorite in this endeavor, and it seems increasingly likely that the best chance

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for more successful diagnosis will rely on a panel of markers that can be used as a powerful adjunct to the existing diagnostic criteria. Any new staging biomarker needs to be cheap, user-friendly, readily applicable to use in the African field, highly, ideally 100%, sensitive and specific, and widely available to clinicians treating patients. If an inexpensive, safe, and orally effective drug for treating late-stage HAT were to become available, then most of these difficult staging issues would be obviated.

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