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Human African trypanosomiasis in Angola: clinical observations, treatment, and use of PCR for stage determination of early stage of the disease

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ABSTRACT

Biological and clinical observations are described for 224 patients infected by human African trypanosomiasis (HAT) in Angola in 2007 and 2008. Seven patients were initially classified in stage 1 (S1), 17 intermediate stage (IS) (WBC <20 lymphocytes/μl with absence of trypanosomes in cerebrospinal fluid (CSF) and no neurological signs), and 200 in stage 2 (S2). Out of 224 patients, 165 (73.6%) presented one or more neurological signs. During treatment with eflornithine, six deaths of S2 patients occurred, five of which were because of an encephalopathy syndrome. Nine patients were diagnosed with a relapse or suspected treatment failure during the follow-up: eight patients after treatment with eflornithine (relapse rate 4.1%) and one patient after pentamidine (6.6%). The contribution of PCR for stage determination evaluated for S1 and IS confirms the difficulty of stage determination, as one S1 patient and two IS patients were carriers of trypanosomes detected a posteriori by PCR in CSF but were treated with pentamidine while follow-up did not confirm treatment efficacy. Since 2001 in Angola, either by passive or active mode detection, approximately 80% of the new cases every year were in S2, whereas the annual number of cases has regressed, probably because the transmission of HAT is decreasing. However, stage determination and treatment remain two major issues for the chronic form of sleeping sickness.

1. Introduction

Human African trypanosomiasis (HAT) occurs in a chronic or acute form. This neglected disease was responsible for devastation in the 1920s, eliminating entire villages. The intervention of mobile teams created by Dr. Eugène Jamot gradually improved the HAT situation until the 1960s. However, a strong recrudescence took place in the 1960s until the beginning of 3rd millennium[1] throughout sub-Saharan Africa, including in Angola.[2] From 2001 to 2009, a reduction in the reported number of new patients was observed, with the Gambian chronic form decreasing from 26 117 cases to 9688, and from 755 to 190 for the rhodesiense acute form.[3] In Angola, one of the countries most affected by the Gambian form with the Democratic Republic of Congo, Chad, and Sudan, a reduction in the number of cases has also been observed every year, from 4577 in 2001 to 295 cases in 2009.

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The chronic form of HAT occurs in Western, Central, and Southern Africa. The causal agent, a trypanosome of the subspecies *Trypanosoma brucei gambiense*, is transmitted to humans by the *Glossina* or tsetse fly, belonging mainly to the species *Glossina palpalis*. The existence of an animal reservoir was suspected, in particular in pigs[4] and wild animals.[5]

In humans, occasionally one of the rare clinical signs of primary infection appears: the chancre of inoculation following the infecting bite by the tsetse. For the chronic form with *T. b. gambiense*, early diagnosis is difficult because of low, fluctuating parasitemia in the blood and the absence of specific clinical signs. After an incubation period (from months to several years),[6] the disease occurs in two stages, the first or hemolymphatic stage (S1) and the second meningoencephalitic stage (S2). Stage 1 is asymptomatic in most cases but can be characterized by a nearly constant fever, nonresponsive to antipyretics, antibiotics and antimalarials, the presence of swollen lymph nodes, cutaneous signs (trypanid, pruritus), edemas of the face or 'lunar facies', splenomegalia, and cardiovascular disorders. After a fluctuating period from months to years, varied central nervous system (CNS) disorders appear, suggesting the S2 stage. This corresponds to the invasion of the CNS by trypanosomes. In endemic HAT areas, the neurological signs suggest trypanosomiasis such as: sleeping disorders, hyperesthesia, motor weakness, strange behavior and gait disturbance. In the absence of treatment, HAT leads to death.

Stage determination depends on two criteria: white blood cell (WBC) count in cerebrospinal fluid CSF (S1 if fewer than five cells/μl); and detection of trypanosomes in CSF by microscopy (S2 if presence of trypanosomes whatever the WBC count).

The WBC cut-off value varies from 5 to 20 lymphocytes/μl. In Angola and Ivory Coast, an intermediate stage (IS) is considered when the WBC ranges from 6 to 19 with absence of trypanosomes in the CSF and with no neurological disorder. The patient is normally treated with pentamidine, like S1 patients,[7] but the final decision regarding the treatment is up to the medical officer, who may consider other criteria leading to treatment of the IS with eflornithine. If the number of cells is higher than five with trypanosomes in the CSF and/or presence of neurological signs, the patient is classified as S2 and treated with eflornithine. It has been shown that molecular biology could improve the determination of S2 by detection of parasite DNA in CSF.[8,9] Given the lack of recent publications on HAT in Angola, this paper summarizes the biological and clinical observations of 224 patients, the contribution of PCR in staging the disease for early stage S1 and IS, and a discussion on the evolution of HAT in Angola since 2001.

2. Methods

2.1. Patients

The study was conducted at the Viana Research and Treatment Center from April 2007 to December 2008. The Viana Center is located 20 km from Luanda, treats only HAT patients, and is directed by the Trypanosomiasis Control Institute of the Ministry of Health. Approximately 20–25 patients were treated at the Viana Center every month during the study period. The disease was detected in consenting patients using active and passive modes according to the traditional method recommended by the WHO[10]: positive using the serological card agglutination for trypanosomiasis test (CATT)/*T. b. gambiense*[11] and detection of the trypanosomes by microscopic examination in lymph node aspirate if presence of swollen cervical lymph nodes, or in blood after concentration of the parasites by centrifugation of capillary tube CTC[12] or miniature anion-exchange centrifugation technique mAECT.[13]

The disease stage was determined by detection of trypanosomes using simple centrifugation of CSF and microscopic examination,[14] WBC count, and clinical examination.

2.2. Treatment and follow-up

Pentamidine was given at a regimen of 4 mg/kg/day IM for 10 days and eflornithine 4 × 100 mg/kg/day IV (every 6 h) for 14 days. Although follow-up is recommended for 24 months, it was recently shown that it was possible to reduce this to 12 months.[15] Follow-ups occurred at 3, 6, 12 and 24 months after treatment. During each follow-up visit, the biological and clinical examinations conducted were identical to those carried out before treatment.

Suspected treatment failure criteria were the following: detection of trypanosomes in CSF after treatment, or WBC count greater than 50 after treatment and higher than the previous counting, or WBC count greater than 20 after treatment and higher than the previous counting with neurological disorders.[16]

2.3. DNA amplification by PCR using cerebrospinal fluid

For the patients in S1 and IS, the search for trypanosome DNA was carried out with PCR using 100 μl of CSF. This molecular investigation was conducted separately, several months after patient admission and treatment, for research purposes only. The mixture of human and parasite DNA was made using the QIAGEN DNA easy purification kit (QIAGEN, Washington, DC, USA). Polymerase chain reaction targeting the microsatellite loci M6C8, MT30/33, and TBDAC was performed according to the protocols published.[8,17] The PCR was not used to improve the detection of treatment failure for technical reason immediately after treatment.

3. Results

3.1. General observations

The 224 patients were detected by passive mode (182, 81.2% at the Viana Center) and active mode (42, 18.8% during medical surveys). Among the 224 patients, 136 were male (60.7%), and a mean age of 32 years (SD 15 years). Twelve patients (5.3%) were diagnosed as relapse after previous treatment with eflornithine (n = 3), melarsoprol (n = 4), and pentamidine (n = 5).