DIAGNOSIS OF HUMAN AFRICAN TRYPANOSOMIASIS

by

N. VAN MEIRVENNE
Institute of Tropical Medicine, Laboratory of Serology,
Nationalestraat 155, B-2000 Antwerpen, Belgium

African trypanosomiasis is an obstinate, life threatening infection killing most of the patients within weeks, months or years. In general, T. b. rhodesiense causes a more fulminant acute disease than T. b. gambiense but the diagnostic approach is essentially the same. During the haemolymphatic stage the trypanosomes invade nearly all parts of the body and sooner or later settle in the central nervous system, marking the onset of the meningoencephalitic stage of the disease. Even then cure remains possible but becomes more and more problematic. Hence, early diagnosis and drug treatment are of vital importance for the individual patient. Moreover, at the epidemiological level, it cuts the parasite's transmission cycle and thus provides a very efficient control strategy. In endemic areas all this incites to active case detection by rural health centres and wherever needed by mobile teams specialized in field surveys.

Six categories of diagnostic criteria and tools can be distinguished: clinical signs and symptoms, bioclinical parameters, parasite, antibody, antigen and DNA detection tests.

Clinical signs and symptoms

Most of the early signs and symptoms are inconstant and aspecific. A transient skin lesion or chancre often develops at the trypanosome inoculation site, particularly in case of T. b. rhodesiense. Another suspicious sign are the trypanids, circular erythema spots that are difficult to see on a black skin. A third suggestive sign are lymph gland swellings, albeit a high percentage of trypanosomized patients will be missed when screening is merely based on gland palpation. Swollen lymph glands, on the other hand, may be present for various other reasons. Several other manifestations may be helpful, especially when they occur in combination: general malaise, irregular fever, headache, tachycardia, hepatosplenomegaly, pain in muscles and joints, pruritus etc. Later neuropathological signs and symptoms are relatively more specific.

Bioclinical parameters

The infection causes various aspecific blood anomalies including lowered haematocrit, increased sedimentation rate and rouleau formation of the red blood cells, decreased serum albumin and increased immunoglobulin levels (IgM in particular), abnormal liver tests, complement and coagulation disorders, presence
of immune complexes and a wide range of bizarre antibodies resulting from autoimmune responses and polyclonal B cell activation.

Parasite detection

The finding of a single trypanosome gives diagnostic certainty but the parasitaemia is often so low that repeated time consuming examination may be required. Examination of chancre scrapings is an uncommon technique. Wet lymph examination is a valuable routine test but has important limitations due to frequent absence of swollen glands in trypanosomiasis patients and presence in others. Experienced palpators possibly feel the difference between "typical" and "atypical" glands but trypanosomes may be present in both! There are many relatively simple techniques for blood examination: wet film, thick smear, capillary buffy coat examination (WOO), mini-anion exchange centrifugation technique (m-AECT). They differ in sensitivity, time needed for reading of the result, cost price and centrifugation requirements. The quantitative buffy coat technique (QBC) is a commercialized promising new tool. The selective haemolytic action of some agents such as SDS can also be exploited to facilitate blood examination. Bone marrow puncture is an exceptional but very sensitive technique. For finding trypanosomes in cerebrospinal fluid a double step centrifugation starting in a tube and ending in a capillary is the most sensitive technique. The same concentration principle can be applied to examine the buffy coat of a large blood sample. Weaning or suckling rats are more or less susceptible to inoculation with *T. b. gambiense* whereas *T. b. rhodesiense* generally also grows in mice. As regards *in vitro* culturing quite promising results have recently been obtained with relatively simple methods.

Antibody detection

Salivarian trypanosomes have a multitude of variable surface antigens (VSG coat) and invariable surface and intracellular antigens. Accordingly, the serum of the patient contains an extremely complex spectrum of anti-trypanosome antibodies that are present in different and fluctuating concentrations. Rigorous selection and standardization of the antigen preparation therefore is a crucial requirement for designing a reliable test. The primary distinction to be made is that between variable and invariable antigens.

Tests with living intact bloodstream form trypanosomes, such as immunolysis and direct agglutination are entirely Variable Antigen Type (VAT) specific. Direct agglutination tests with fixed procyclic (PAT) or bloodstream form trypanosomes (CATT) detect antibodies to either invariable or invariable and variable surface antigens. In most indirect immunofluorescence test procedures intracellular antigens interact as well. Numerous test systems (indirect latex or red blood cell agglutination (IHA), precipitation, ELISA etc.) make use of crude or purified trypanosome antigens. A breakthrough is to be expected from the introduction of perfectly defined recombinant or synthetic antigens. Recently an innovative assay detecting antibodies to parasite enzymes has been introduced.

Reliable laboratory and field tests, including several systems using defined variable antigens, already exist for *T. b. gambiense* and have proven indispens-
able tools for mass surveys. Similar tests allowing even earlier detection of *T.b. rhodesiense* infections remain to be developed.

Most antibody tests can be applied to serum, plasma, wet or dry blood and cerebrospinal fluid. If needed sensitivity and specificity of the assay can be adjusted to some extent by varying dilutions, conjugates and cut-off values. False negative results are to be expected at a very early stage of infection. False or unexplainable positives may be due to cross-reactions (malaria may be a complicating factor!), unknown history of cured trypanosomiasis or abortive infections with other trypanosome species.

Those making large-scale use of antibody detection tests for mass screening should be aware of the classical formula for calculating, in terms of percentage, the predictive value of a positive (PV+) and negative (PV-) result. These PVs are determined by three variables, all in terms of percentage: prevalence of the infection and sensitivity and specificity of the test system. In general, a test endowed with high specificity (99% looks possible) and somewhat lower sensitivity (95% would be excellent) is indicated for mass screening. If prevalence is 1% the PV- than reaches 99.9% and the PV+ or true seropositives nearly 49%. At a prevalence of only 0.1% the PV- still exceeds 99% but the PV+ drops to 8.7%, implying that 91.3% false seropositives would be submitted to laborious parasitological examination without avail. These data of course also raise questions about cost benefit ratios and about passive versus active surveillance strategies to be applied under different circumstances.

Antigen detection

Unlike antibody the presence of circulating specific trypanosomal antigens is a certain indication of an ongoing or recently cured infection. A first generation of antigen detection tests already exists. These are mainly ELISA systems using polyclonal or monoclonal antibodies to one or more invariable intracellular or surface antigens. Simple latex agglutination tests are also being developed and there is good hope that excellent field assays will become available to complement parasite and antibody detection tests.

DNA detection

The idea of developing a sensitive test for detection of specific trypanosomal DNA has proven fully realistic. Probably it will become PCR amplification assays using non radioactive probes complementary to some nuclear or kinetoplast DNA sequences. No doubt we are dealing here with most powerful tools for epidemiological research but the applicability to field surveys aiming at individual diagnosis and treatment remains questionable.

Drug treatment and follow-up

Once the infection detected in lymph or blood the cerebrospinal fluid has to be examined for markers characterizing the meningoencephalitic stage of the disease. This is a most delicate step in the diagnostic procedure having decisive consequences for the chemotherapeutic agents and regimens to be applied. The mere finding of a few trypanosomes in the CSF is no conclusive indication for
involvement of the central nervous system. More reliable parameters are the increase of white blood cells, total protein, albumin and particularly the presence of IgM and anti-trypansomosome antibodies. IgM levels can be estimated with a commercial latex agglutination test and similar tests could be developed for other markers.

Efficacy of therapy is primarily assessed by definitive disappearance of the parasite, the judging of which would be much facilitated by reliable antigen or DNA detection tests. Antibody titers decrease very slowly and complete negativation may take several months or even years. Cure of patients with CNS involvement is reflected by progressive normalization of the CSF. For the moment there is no method to distinguish between relapse and reinfection.