Confirmation of antibodies against L-tryptophan-like epitope in human African trypanosomosis serological diagnostic

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Antibodies directed against L-tryptophan epitope (WE - W for tryptophan, E for epitope), a constant epitope borne by variant surface glycoproteins (VSG), have been detected in sera of all 152 Human African Trypanosomosis (HAT) patients from Angola. The WE is present in VSG hydrophobic regions of the C terminal domains. In the assay, L-tryptophan was linked to bovine serum albumin (BSA) with glutaraldehyde to synthesize W-G-BSA conjugate which was used in an enzyme-linked immunosorbent assay (ELISA) to detect the antibodies. A significant difference was found between HAT patients and controls confirming previous results obtained with a lower number of patients in Congo. A diagnostic test based on this synthetic epitope, especially in combination with other tests, might improve the HAT diagnostic test in field conditions.

Key words: Tryptophan, enzyme-linked immunosorbent assay (ELISA), human African trypanosomosis, serological diagnostic.

INTRODUCTION

Trypanosoma brucei gambiense (T. b. gambiense) and T. b. rhodesiense, the causative agents of Human African Trypanosomosis (HAT) or sleeping sickness, are tsetse fly-transmitted protozoan parasites. Diagnosis of HAT
remains a challenge for disease control as HAT is characterized by a non-specific clinical presentation and its definitive diagnosis requires trypanosome detection by microscopy (WHO, 2013). Additionally, the detection of this parasite is difficult because of the low parasite concentration in circulating blood, despite the use of concentration methods such as the mini-anion-exchange centrifugation technique (mAECT) (Lumsden et al., 1979). Moreover, molecular diagnostics based on parasite DNA or RNA detection are more sensitive but are not adapted to field conditions.

Serological diagnostic test of HAT, caused by *T. b. gambiense*, had been performed in the field during medical surveys since 1978 using the Card Agglutination Test with stained Trypanosomes (CATT) (Magnus et al., 1978). CATT/*T. b. gambiense* is still the reference test in the field conditions although, it requires refrigeration and an electric rotator, with drawbacks of false positive and negative results (Magnus et al., 1978). Trypanosomes produce a set of variant surface glycoproteins (VSG). Thus, new diagnostic approaches are based using native surface glycoproteins although the variable nature of the VSG is not satisfactory for a serological diagnostic test (WHO, 2013).

Following an initiative of Foundation for Innovative New Diagnostics (FIND), a rapid diagnostic test is now available (Standart Diagnostic SD Bioline HAT, Sternberg et al., 2014). However, this latter is very useful for passive mode detection but not for mass screening survey (Büscher et al., 2014). The search of new biomarker candidates will permit an improvement to HAT diagnostic test based on CATT (Bonnet et al., 2015). Proteomic investigations are identifying potential immunodiagnostic parasite protein antigens and several biomarkers that are in preliminary evaluation or design for new diagnostic tests (Holzmuller et al., 2013). It has also been discussed how molecular diagnostics may contribute to the elimination of HAT (Büscher and Deborggraeve, 2015).

A tryptophan-like epitope (WE) was found in VSGs from the *T. brucei* group (Semballa et al., 2007). The tryptophan residue is one of the conserved flanking residues of cysteine residues in their hydrophobic regions in the C terminal domain. Antibodies directed to WE were previously found in the sera of HAT patients detected during medical survey (active mode detection), but they were not detected in HIV infection, malaria, Chagas and Parkinson’s diseases (Okomo-Assoumou et al., 1995). This latter investigation was performed in the Bouenza focus in the Republic of Congo. No other study has been done in a different HAT focus.

In the present study, anti-WE detection in sera of HAT patients from Angola detected by passive mode at the Viana hospital compared to negative controls, in order to confirm the potential value of this immune marker in another endemic country, use in setting up a new test for serological HAT mass screening.

**METHODS**

**Patients**

Informed consent was obtained from all individual participants which were included in the study. Patients were diagnosed by passive mode detection in Viana hospital in Angola (Truc et al., 2012). Only CATT positive patients with trypanosomes that were detected in one body fluid (blood, lymph juice, cerebrospinal fluid) were selected. Stage determination of the disease was done by searching trypanosomes and white blood cells count in CSF: 13 in stage 1 (early period) and 139 in stage 2 (neurological period). A clinical examination allows detection of neurological signs. Serum from CATT negative subjects living in the same endemic area were used as controls. These controls were uninfected volunteer persons. Serum aliquots were kept at -80°C until use.

**ELISA based on L-tryptophan conjugates**

Enzyme-linked immunosorbent assay (ELISA) was performed as previously described (Okomo-Assoumou et al., 1995). Briefly, W and BSA (bovine serum albumin) were dissolved in 1.5 M acetate buffer, pH 8 before the addition of glutaraldehyde. After dialysis, the conjugate (W-G-BSA) or control (G-BSA) was added into polystyrene well plate. The well plate was filled with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-Tween), 10% glycerol and BSA (5 g/L). The well plate was incubated for 1 hr at 37°C to saturate it. The plate was rinsed twice with PBS-Tween. The well plate was then filled with 200 µL of diluted (2,000-fold) serum plus PBS-Tween containing BSA (5 g/L) and 10% glycerol. After washings, horseradish peroxidase-conjugated goat antibodies were added to human immunoglobulins. Thereafter, substrate solution and stop solution were successively added. The absorbance in the well plate was measured at 492 nm. The specific immunologic binding of sera was obtained by subtracting blank values read on well plates coated with BSA-G from experimental absorbance values.

**Statistical method**

Results are expressed in OD value for each individual. Comparison between the patients and the control groups was made using the nonparametric Wilcoxon–Mann–Whitney test with median and quartiles. The difference was considered significant when *p*<0.05. This comparison was illustrated by a graph performed with Graph Pad V6.0.

**RESULTS AND DISCUSSION**

Anti-WE antibodies were detected in serum of 152 patients (Table 1) and in 10 controls (Table 2). The limited
Table 1. Values of anti-WE antibodies in optic density and clinical stage for each patient.

| Patient Code | Stage | OD Units (anti-WE) | Patient Code | Stage | OD Units (anti-WE) |
|--------------|-------|--------------------|--------------|-------|--------------------|
| V31          | 1     | 0.546              | V117         | 2     | 0.750              |
| V34          | 1     | 0.475              | V119         | 2     | 0.725              |
| V36          | 1     | 0.536              | V122         | 2     | 3.808              |
| V38          | 1     | 0.442              | V124         | 2     | 0.546              |
| V40          | 1     | 0.291              | V125         | 2     | 1.346              |
| V42          | 1     | 0.447              | V128         | 2     | 0.722              |
| V91          | 1     | 0.345              | V129         | 2     | 0.635              |
| V198         | 1     | 0.404              | V130         | 2     | 0.451              |
| V201         | 1     | 0.225              | V133         | 2     | 0.716              |
| V203         | 1     | 0.320              | V134         | 2     | 0.676              |
| V215         | 1     | 0.259              | V135         | 2     | 0.602              |
| V217         | 1     | 0.382              | V137         | 2     | 0.593              |
| V131         | 1     | 0.267              | V138         | 2     | 0.604              |
| V32          | 2     | 0.500              | V139         | 2     | 0.505              |
| V63          | 2     | 0.514              | V141         | 2     | 0.592              |
| V120         | 2     | 0.371              | V142         | 2     | 0.779              |
| V231         | 2     | 0.540              | V143         | 2     | 0.780              |
| V02          | 2     | 0.311              | V144         | 2     | 0.736              |
| V03          | 2     | 1.317              | V145         | 2     | 0.634              |
| V05          | 2     | 1.848              | V146         | 2     | 0.592              |
| V07          | 2     | 0.345              | V147         | 2     | 1.288              |
| V10          | 2     | 1.251              | V150         | 2     | 0.707              |
| V13          | 2     | 0.628              | V151         | 2     | 0.852              |
| V15          | 2     | 0.772              | V152         | 2     | 0.509              |
| V16          | 2     | 0.914              | V153         | 2     | 0.724              |
| V17          | 2     | 0.730              | V156         | 2     | 0.777              |
| V18          | 2     | 0.911              | V157         | 2     | 0.557              |
| V19          | 2     | 1.107              | V159         | 2     | 0.691              |
| V23          | 2     | 0.792              | V160         | 2     | 0.504              |
| V24          | 2     | 0.693              | V162         | 2     | 0.622              |
| V25          | 2     | 0.426              | V164         | 2     | 0.871              |
| V26          | 2     | 0.598              | V165         | 2     | 0.602              |
| V27          | 2     | 0.733              | V166         | 2     | 0.512              |
| V35          | 2     | 0.554              | V167         | 2     | 1.234              |
| V37          | 2     | 0.683              | V168         | 2     | 0.730              |
| V43          | 2     | 0.783              | V170         | 2     | 0.760              |
| V44          | 2     | 0.784              | V171         | 2     | 0.715              |
| V46          | 2     | 1.036              | V172         | 2     | 0.850              |
| V47          | 2     | 0.775              | V173         | 2     | 0.530              |
| V48          | 2     | 0.687              | V174         | 2     | 0.517              |
| V49          | 2     | 0.588              | V176         | 2     | 0.642              |
| V52          | 2     | 0.655              | V177         | 2     | 0.736              |
| V53          | 2     | 0.603              | V178         | 2     | 0.546              |
| V54          | 2     | 0.736              | V179         | 2     | 0.794              |
| V57          | 2     | 0.856              | V180         | 2     | 0.577              |
| V61          | 2     | 0.915              | V182         | 2     | 0.607              |
| V64          | 2     | 0.728              | V183         | 2     | 1.055              |
| V65          | 2     | 0.664              | V187         | 2     | 0.573              |
| V66          | 2     | 0.582              | V188         | 2     | 0.719              |
| V67          | 2     | 0.652              | V189         | 2     | 0.746              |
| V69          | 2     | 0.659              | V191         | 2     | 0.700              |
number of the control group was due to the difficulty in obtaining the people's consent at the Viana Hospital. In a survey carried out in Congo, including 22 controls and 76 patients (52 and 24 in stages 1 and 2 respectively), a very low immunological signal was detected in controls.

The comparison between patients (median= 0.6622, n=152) and controls values is highly significant P <0.0001 (Figure 1). This confirms the use of anti-WE as a potential biomarker in HAT serological diagnostic test. Further investigations are required to compare the present results with the post-cure HAT persons and other parasitic diseases. Furthermore, among 139 stage 2 patients, 86 presented neurological disorders. There is no correlation between OD values and presence of neurological disorders P=0.58. Thus, the anti-WE value is not correlated to patient clinical state.

Rapid tests are now available and are very promising but have been employed mainly for passive mode detection (Büscher et al., 2014). However, their observed specificity was relatively low. A larger study on their use in field condition in combination and in comparison with immune trypanalysis, the reference test, is under progress.

L-Tryptophan epitope conjugate production by peptide synthesis is now cheaper and easily compared to CATT antigen production, requiring T. b. gambiense infection in laboratory rodents, or production of recombinant proteins to develop diagnostic kits. Moreover, WE conjugate is thermo-stable and can be developed in a rapid test format. For instance, as most anti-WE antibodies are of IgM isotype, an agglutination assay is feasible. Reliably and easy to use, diagnostic tools are necessary to eliminate HAT as a public health problem in the year 2020 and zero transmission in the year 2030.

### Table 2. Values of anti-WE antibodies in optic density for each negative control individual.

| Control Code | OD Units (anti-WE) |
|--------------|--------------------|
| AG           | 0.109              |
| AR           | 0.108              |
| EB           | 0.096              |
| MR           | 0.114              |
| NF           | 0.089              |
| BA           | 0.111              |
| BH           | 0.192              |
| MF           | 0.121              |
| OE           | 0.117              |
| OC           | 0.110              |
Figure 1. Level of anti-WE antibodies, as assayed by ELISA, in the HAT patients and control groups. Black lines indicates median and interquartiles (Q25 and Q75).

Conflict of Interests

The authors have not declared any conflict of interests.

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