Low specificities of HIV diagnostic tests caused by *Trypanosoma brucei gambiense* sleeping sickness

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Abstract

BACKGROUND: Accuracy of HIV diagnostic tests in tropical infections is poorly documented. Human African trypanosomiasis (HAT) is characterized by a polyclonal B-cell activation, constituting a risk for false positive reactions in diagnostic tests, including HIV tests.

METHODS: A retrospective HIV diagnostic test accuracy study was performed on 360 human African trypanosomiasis (HAT) patients infected with *T.b. gambiense* before treatment, and 163 patients 2 years after successful treatment in Mbuji Mayi, East Kasai, DR Congo. Sensitivity, specificity and positive predictive value (PPV) of individual tests and algorithms consisting of 3 rapid tests were determined.

RESULTS: Sensitivity for all tests was 100% (11/11). Low specificity (96.3%, 335/348) and PPV (45.8%, 11/24) of a classical seroconfirmation strategy (Vironostika ELISA followed by Line Immunoassay) complicated determination of the HIV status, which had to be determined by PCR. Specificities of rapid diagnostic tests were 39.1% for Determine (136/348), 85.3-92.8% (297/348-323/348) for VIKIA, Immunoflow, Doublecheck and Bioline, and 96.6-98.3% (336/348-342/348) for UniGold, Oraquick and STAT-PAK. Specificity for Vironostika was 67.5% (235/348). PPVs ranged between 4.9 and 64.7%. Combining 3 different rapid tests resulted in specificities of 98.3-100% (342-348/348) and PPVs of 64.7-100% (11/17-11/11). In cured HAT patients, specificities were significantly higher for Vironostika, Determine, Unigold and Immunoflow.

CONCLUSIONS: *T.b. gambiense* infection decreases the specificity of antibody detection tests for HIV diagnosis. Unless tests have been validated for interference with HAT, HIV diagnosis in untreated HAT using classical algorithms should be avoided. Specific, validated combinations of 3 HIV rapid tests can increase specificity.

Introduction

Over 33 million people live with HIV worldwide, of which two-thirds are in sub-Saharan Africa [16]. Rapid diagnostic tests (RDT) are increasingly used for HIV diagnosis. In 2007, the proportion of RDTs among diagnostic tests procured through World Health Organization (WHO) increased to 96% and the majority were destinated to Africa [19]. Accuracy of HIV diagnostic tests in persons suffering from common infections in sub-Saharan Africa is poorly documented. In Tanzania, false positive HIV ELISA results were associated with diagnosis of urinary schistosomiasis and high rheumatoid factor titers [3]. Performance of HIV enzyme immunoassays may be unsatisfactory in visceral leishmaniasis and uncomplicated malaria, resulting in poor positive predictive values [4;12;14]. These false positive results in HIV antibody detection tests have been explained by the polyclonal B-cell activation in visceral leishmaniasis and malaria [4;14].

Human African trypanosomiasis (HAT) or sleeping sickness is a fatal disease caused by the protozoan parasites *Trypanosoma brucei (T.b.) gambiense* and *T.b. rhodesiense*, and is transmitted by tsetse flies. In 36 HAT endemic countries in sub-Saharan Africa, about 11000 new cases are diagnosed yearly, on a screened population of 2 to 3 million [15]. Polyclonal B-cell activation is also observed in HAT [5]. The uncontrolled antibody production constitutes a risk for false positive reactions in diagnostic tests, including HIV tests, but has hardly been studied, although antibodies cross-reacting with other pathogens have been reported [1;2;13].

We assessed the accuracy of commonly used diagnostic tests detecting antibodies against HIV in patients suffering from *gambiense* human African trypanosomiasis and in the cured subgroup of these patients two years after treatment.

Materials and methods

Patients

360 human African trypanosomiasis patients infected with *T.b. gambiense* participated to a longitudinal study for monitoring clinical outcomes after HAT treatment, conducted in the hospital of Mbuji Mayi, Kasai Province, DR Congo. Patients and study outcomes are described elsewhere [9]. Patients were prospectively enrolled using the following inclusion criteria 1° trypanosomes in lymph node aspirate, blood or CSF; 2° ≥12 years old; and 3° living within a 100 km perimeter around Mbuji Mayi. Exclusion criteria were: 1° pregnancy; 2° no guarantee for follow-up; 3° moribund condition; 4° hemorrhagic CSF; 5° concurrent serious illness like tuberculosis, bacterial or cryptococcal meningitis. At time of inclusion, no information was available regarding HIV status. Patients were treated for HAT according to the guidelines of the national program for sleeping sickness of DR Congo. Seventeen patients died during treatment, 14 died during follow-up from non-HAT related reasons, 165 patients experienced treatment failure and 32 did not attend the 24 months visit [9]. Cure of HAT, assessed at 24 months post-treatment, was achieved in 163/360 patients. The Ministry of Health, DR Congo, and the Ethical Committee of the University of Antwerp, Belgium, approved the protocol as well as an amendment allowing for HIV testing. We report on results of HIV tests performed retrospectively at the Institute of Tropical Medicine in Antwerp, on specimens taken before HAT treatment and, for 163 cured patients, also at 24 months after HAT treatment.

Specimens

Blood taken on clotting activator was allowed to clot for 1 hour at ambient temperature and centrifuged at 1000g for 15 minutes. Serum was collected and immediately frozen in liquid nitrogen, shipped on dry ice and stored at -80°C until use.

Blood taken on heparin was mixed with an equal volume of AS1 storage buffer (Qiagen, Germany). Specimens were shipped and stored at ambient temperature. DNA was extracted with the QIAamp DNA blood mini kit (Qiagen, Germany).

Reference HIV tests

Serum specimens were screened for presence of HIV specific antibodies and antigens using the Vironostika HIV Uni-Form II Ag/Ab ELISA test (bioMérieux, Boxtel, The Netherlands). If the result of Vironostika was reactive (optical density>cut-off) and taking into account a grey zone (cut-off-20%<optical density<cut-off), serum was tested with the INNO-LIA™ HIV I/II Score line immuno-assay (Innogenetics, Ghent, Belgium). Following the instructions of the manufacturer, INNO-LIA results were interpreted as negative, positive or indeterminate. INNO-LIA was followed by the INNOTEST® HIV Antigen mAb ELISA (Innogenetics, Ghent, Belgium) to detect early seroconversions. To confirm or exclude HIV infection in certain cases, HIV PCR was performed, following a nested method in an algorithm of three different primer sets in pol, env and LTR region. The obtained sensitivity and specificity of this PCR is 100%. [17].

Rapid diagnostic tests for detection of HIV specific antibodies

All serum specimens were tested in Determine™ HIV 1/2 (Inverness Medical, Unipath Ltd. Bedford, United Kingdom), Uni-Gold™ HIV (Trinity Biotech plc, Bray, Co Wicklow, Ireland), OraQuick Rapid HIV-1/2 Antibody Test (Orasure Technologies Inc., Bethlehem, PA USA), DoubleCheckGold™ HIV 1&2 (Orgenics, Yavne, Israel), SD Bioline HIV-1/2 3.0

(Standard Diagnostics Inc., Yongin-si, Kyonggi-do, Korea), ImmunoFlow HIV1-HIV2 (Core Diagnostics, Birmingham, United Kingdom), VIKIA® HIV 1/2 (bioMérieux, Marcy-L'Etoile, France) and HIV 1/2 STAT-PAK assay (Chembio Diagnostic systems Inc, Medford, NY USA), following the instructions of the manufacturers.

Data analysis

2x2 tables were constructed. STATA version 10 was used to calculate specificities and positive predictive values (PPV) with 95% confidence intervals (CI). Specificities were compared using the McNemar chi square test.

Results

HIV status of HAT patients before treatment

Stored serum was available for 359/360 HAT patients. Determination of the HIV status of the HAT patients was unexpectedly complicated. In Vironostika, 25/359 specimens were classified as grey zone and 99/359 as reactive. In INNO-LIA, 51/124 of these specimens tested indeterminate and 26/124 were positive. Eleven INNO-LIA positive sera reacted strongly with all HIV-1 test lines (sgp120, gp41, p31, p24, and p17). The remaining 15 INNO-LIA positive sera, reacted weakly and had unusual, incomplete HIV-1 line combinations (no reaction with p31, p24, or p17 in 8-14 samples). To detect possible seroconverters, all 113 specimens that were Vironostika grey zone or reactive and INNO-LIA negative, indeterminate or weakly positive, were tested in INNOTEST® HIV Antigen, and were negative. Facing an unusual high number of unclear results with the classical serological confirmation strategy, PCR was performed on INNO-LIA positives to solve the specificity problem. Only the 11 strong INNO-LIA positives were confirmed to be HIV positive in PCR.

Thus, HIV prevalence in HAT patients was 3.1% (11/359, CI 1.5-5.4%). Specificity of Vironostika was 67.5% (235/348, CI 62-72%) with a positive predictive value (PPV) of 8.9% (CI 4.5-15.3%). Considering samples HIV positive if both Vironostika reactive and INNO-LIA positive, specificity and PPV of the classical serological confirmation strategy were respectively 96.3% (335/348, CI 94.2-98.3%) and 45.8% (11/24 CI 25.4-66.3%).

Results of HIV RDT in HAT patients before treatment

Sensitivity of all individual rapid tests was 100% (11/11). Specificities (table 1) were 39.1% for Determine, 96.6% for UniGold, 98.0% for Oraquick, 91.7% for Doublecheck, 92.8% for

Bioline, 90.2% for Immunoflow, 85.3% for VIKIA and 98.3% for STAT-PAK. Positive predictive values (table 1) ranged from 4.9 to 64.7%.

False positivity seemed randomly scattered with one out of 348 HIV negative samples reactive in 6 RDTs, 2/348 in 5 RDTs, 6/348 in 4 RDTs, 17/348 in 3 RDTs, and 63/348 HIV negative samples reactive in 2 RDTs.

HIV diagnosis in low prevalence settings should be based on the combination of 3 different RDT's, considering only those patients HIV positive if 3 assays are reactive. All test algorithms consisting of 3 different RDTs result in 100% sensitivity, specificities ranged between 98.3% (6 false positives, 342/348, 96.9-100%) and 100% (348/348). Seventeen specific three-test combinations provided 100% specificity (Table 2).

HIV status of cured HAT patients, 2 years after HAT treatment

Two years after treatment for HAT, 163/360 patients were declared cured from HAT. From 162, serum was available. Among these 162 patients, one was HIV positive before HAT treatment, and was reconfirmed by HIV PCR two years post-treatment. Amongst the 161 remaining samples, 4 were grey zone or reactive in Vironostika and indeterminate or positive in INNO-LIA. They were INNOTEST® HIV Antigen and PCR negative. Thus all 161 HIV pre-treatment negative patients remained HIV negative. Specificity of the Vironostika test increased significantly (Table 3).

Results of HIV RDT in cured HAT patients

After cure from HAT, the HIV positive patient remained reactive in all HIV RDTs. Specificity increased significantly for Determine, Unigold and Immunoflow tests and was higher but not significant for the Oraquick, Bioline, VIKIA and STAT-PAK tests (table 3). For Doublecheck, specificity was significantly lower on specimens from cured patients.

Discussion

When testing *T.b. gambiense* HAT patients, low specificities were observed in antibody detection RDTs as well as in reference tests (ELISA and Line ImmunoAssay) for HIV diagnosis. On cured HAT patients, specificities were higher. Sleeping sickness thus decreases the specificity of HIV diagnostic antibody detection tests, including tests and algorithms used for confirmation.

Test specificities of HIV RDTs observed in HAT patients are below specificities of ≥99% reported by WHO [18]. Surprisingly, this is the first report on low specificity of HIV tests in *T.b. gambiense* HAT. Other studies examining HIV prevalences in HAT did not report specificity problems of the second generation ELISA and Western Blot tests used [7;8;10;11]. False positive results of HIV ELISAs were observed in uncomplicated malaria, with specificities of 96.4 and 98.4% and positive predictive values of 53 and 57% [4] and visceral leishmaniasis with 9% of false positive results [14]. Urinary schistosomiasis and rheumatoid factor were associated with false positive HIV test results [3]. Although *T.b. gambiense* is not the only protozoan parasite impairing HIV test specificity, its effect seems more profound. This might be due to the strong non-specific polyclonal B-cell activation and/or high concentration of rheumatoid factor-like anti-immunoglobulin antibodies occurring in sleeping sickness [5;6]. The specificity differences between tests might be caused by different antigens, or different involvement of IgM. To increase sensitivity, several third generation tests include IgM in the reaction. Such HIV tests are at higher risk for non-specific reactions, as antibodies resulting from the B-cell activation in HAT belong mainly to the IgM class [5].

In the absence of an endemic HAT negative control group, the relationship between the low test specificity and HAT could be demonstrated by comparing pre-and post-HAT treatment specificities in the same patients. Presence of other immunological disorders or biological

factors such as co-infections in our HAT patients were not examined. Although we cannot exclude their interference in the performance of the HIV tests, this seems unlikely since the same tests were repeated in the same persons after cure from HAT. The overall specificities of HIV RDTs in the region remain unknown, although they are expected to be equal or higher than the observed specificities in cured HAT. The accuracy of DoubleCheckGold™ HIV 1&2 should be further investigated, since we have no explanation for its decrease in specificity after successful treatment for HAT, despite use of the same test batch number.

Considering the difficulties we encountered at the reference laboratory in determining the real HIV status of HAT patients, it is obvious that the risk for wrong diagnosis is quite high. Not only is there the risk of false positive HIV diagnosis in patients with confirmed HAT, but also of HAT being misdiagnosed as HIV, leaving the HAT patient without treatment. Confounding HAT and HIV diagnosis may become particularly problematic in the context of integration of HAT control in basic health infrastructures [15]. Clinicians easily oversee HAT if they are not familiar with the disease, since the clinical picture may mimic malaria, tuberculosis, toxoplasmosis, viral encephalitis, brucellosis, lymphoma, typhoid fever, neurosyphilis, HIV and opportunistic infections (e.g. cryptococcosis, toxoplasmosis). One should be careful to generalize our observations since we haven't tested the accuracy of HIV antibody tests in *T.b. rhodesiense* HAT patients.

Our results support the recommendation by WHO to perform 3 different RDTs for HIV diagnosis in settings with prevalences lower than 10% [18]. Considering only those persons HIV positive if they are reactive in 3 different tests, increases the total specificity of HIV RDTs in HAT, although up to 1.7% of false positivity remained. Although we report on specificities of different RDTs and of test algorithms for HIV diagnosis in *T.b. gambiense* HAT patients, we withhold from proposing an optimal test algorithm for *T.b. gambiense* endemic regions. In the present study, the number of HIV positive samples was too low to

make reliable estimations of test sensitivities. Performance of the various assays, both reference tests and RDT, remains to be determined in a HAT negative population. Moreover, besides test sensitivity and specificity other factors influence the choice of the most suitable test algorithm, such as cost, test availability, robustness, user friendliness, test rapidity and necessary equipment.

In conclusion, *T.b. gambiense* infection decreases the specificity of antibody detection tests for HIV diagnosis. Even a gold standard seroconfirmation strategy does not provide a solution for determining the real HIV status, and limited data on the performance of a Western blot test also show lower specificity caused by indeterminate results. Since the risk for HIV misdiagnosis is considerable, HIV diagnosis should not be established in the setting of an untreated HAT infection using classical algorithms, unless the applied tests have been validated specifically for non-interference with HAT. Although a risk for false positive reactions in HAT remains, specific combinations of 3 different serially applied HIV RDTs increase specificity and should be applied for determination of the HIV status.

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308 Table 1: Specificity and positive predictive value (PPV) with 95% confidence intervals (CI)
309 observed with diagnostic HIV tests in 348 untreated human African trypanosomiasis patients
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	Pre-treatment specificity n/N (%, 95% CI)	PPV n/N (%, 95% CI)
Bioline	323/348 (92.8%, 90.1-95.5%)	11/36 (30.6%, 15.2-45.9%)
Determine	136/348 (39.1%, 33.9-44.2%)	11/223 (4.9%, 2.1-7.8%)
DoubleCheck	319/348 (91.7%, 88.7-94.6%)	11/40 (27.5%, 13.4-41.6%)
ImmunoFlow	314/348 (90.2%, 87.1-93.4%)	11/45 (24.4%, 11.7-37.2%)
Oraquick	341/348 (98.0%, 96.5-99.5%)	11/18 (61.1%, 37.9-84.4%)
STAT-PAK	342/348 (98.3%, 96.9-100%)	11/17 (64.7%, 41.2-88.2)
Unigold	336/348 (96.6%, 94.6-98.5%)	11/23 (47.8%, 26.9-68.8%)
VIKIA	297/348 (85.3%, 81.6-89.1%)	11/62 (17.7%, 8.1-27.4%)
Vironostika	235/348 (67.5%, 62.6-72.5%)	11/124 (8.9%, 3.8-13.9%)

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313 Table 2: Combinations of 3 different HIV rapid diagnostic tests providing 100% specificity in
314 348 untreated *T.b. gambiense* patients. Only patients that were reactive in all 3 RDTs were
315 considered HIV positive. The order in which test combinations are presented is alphabetical,
316 reflects parallel testing and is no suggestion for test order in serial testing.

317

Bioline + Determine + STAT-PAK
Bioline + Immunoflow + Oraquick
Bioline + Oraquick + STAT-PAK
Bioline + STAT-PAK + Unigold
Determine + Doublecheck + STAT-PAK
Determine + Immunoflow + STAT-PAK
Determine + STAT-PAK + VIKIA
Doublecheck + Immunoflow + Oraquick
Doublecheck + Immunoflow + Unigold
Doublecheck + Oraquick + STAT-PAK
Doublecheck + STAT-PAK + Unigold
Immunoflow + Oraquick + STAT-PAK
Immunoflow + Oraquick + Unigold
Immunoflow + STAT-PAK + Unigold
Immunoflow + Unigold + VIKIA
Oraquick + STAT-PAK + VIKIA
STAT-PAK + Unigold + VIKIA

318

319 Table 3: HIV ELISA and RDT test results in 161 HIV negative HAT patients, before
320 treatment for HAT (pre-treatment) and 2 years after successful treatment (post-treatment).
321 Specificities were compared using the McNemar chi square test.

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	Pre-treatment specificity n/N (%, 95CI)	Post-treatment specificity n/N (%, 95CI)	*p*
Bioline	150/161 (93.2%, 89.2-97.1%)	154/161 (95.7%, 92.5-98.8%)	0.25
Determine	74/161 (46.0%, 38.2-53.7%)	139/161 (86.3%, 81.0-91.7%)	<0.001
DoubleCheck	148/161 (91.9%, 87.7-96.2%)	130/161 (80.7%, 74.6-86.9%)	0.003
ImmunoFlow	147/161 (91.3%, 86.9-95.7%)	155/161 (96.3%, 93.3-99.2%)	0.046
Oraquick	158/161 (98.1%, 96.0-100%)	160/161 (99.4%, 98.2-100%)	0.16
STAT-PAK	158/161 (98.1%, 96.0-100%)	160/161 (99.4%, 98.2-100%)	0.3
Unigold	155/161 (96.3%, 93-99.2%)	160/161 (99.4%, 98.2-100%)	0.03
VIKIA	134/161 (83.2%, 77.4-89.1%)	144/161 (89.4%, 84.6-94.2%)	0.08
Vironostika	119/161 (73.9%, 67.1-80.8%)	158/161 (98.1%, 96.0-100%)	<0.001

323