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CARD AGGLUTINATION TEST FOR TRYPANOSOMIASIS (CATT) END-DILUTION TITER AND CEREBROSPINAL FLUID CELL COUNT AS PREDICTORS OF HUMAN AFRICAN TRYPANOSOMIASIS (*TRYPANOSOMA BRUCEI GAMBIENSE*) AMONG SEROLOGICALLY SUSPECTED INDIVIDUALS IN SOUTHERN SUDAN

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Abstract. The diagnosis of human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* relies on an initial serologic screening with the card agglutination test for trypanosomiasis (CATT) for *T. b. gambiense*, followed by parasitologic confirmation in most endemic areas. Unfortunately, field parasitologic methods lack sensitivity and the management of serologically suspected individuals (i.e., individuals with a positive CATT result but negative parasitology) remains controversial. In Kajo-Keji County in southern Sudan, we prospectively collected sociodemographic and laboratory data of a cohort of 2,274 serologically suspected individuals. Thirty-three percent (n = 749) attended at least one follow-up visit and HAT was confirmed in 64 (9%) cases. Individuals with lower initial CATT-plasma (CATT-P) end-dilution titers had lowest risks (10.4 and 13.8/100 person-years for 1:4 and 1:8 titers, respectively) that significantly increased for higher dilutions: relative risks = 5.1 (95% confidence interval [CI] = 2.6–9.5) and 4.6 (95% CI = 2.8–9.8) for 1:16 and 1:32 titers, respectively. The cumulative yearly risk was also high (76%) in individuals found with 11–20 cells in the cerebrospinal fluid, but this involved only eight patients. Adjustment for potential confounders did not affect the results. In conclusion, treatment with pentamidine should be considered for all serologically suspected individuals with a CATT-P end-dilution titer ≥ 1:16 in areas of a moderate to high prevalence of HAT.

INTRODUCTION

Field diagnosis of human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* relies on the initial screening with the card agglutination trypanosomiasis test (CATT/*T. b. gambiense*) in most endemic areas. The CATT/*T. b. gambiense* is a cheap, quick, and practical serologic test that has been widely used since it was developed in 1978.[1] It is a highly sensitive test when applied on undiluted whole blood (CATT-WB).[2] Despite its good specificity, the positive predictive value (PPV) of the CATT-WB is limited because the test is used for mass screening of populations in which the prevalence of HAT rarely exceeds 5%.[2,3] The specificity of the CATT can be further improved when performed on plasma (CATT-P) or serum diluted to 1:4,[4] but this is still insufficient. Confirmatory diagnosis thus relies on the finding of trypanosomes in the lymph nodes, blood, or cerebrospinal fluid (CSF). However, despite the use of concentration methods to detect trypanosomes in the blood or CSF, the field parasitologic methods have a limited sensitivity and fail to detect all infected individuals.[5,6]

In Kajo-Keji County in southern Sudan, Médecins Sans Frontières has been conducting since June 2000 a sleeping sickness control program that relies on the systematic screening of the entire county population and treatment of all infected patients. The main objective of the program is to decrease the prevalence of HAT to less than 0.5% in all villages. Individuals with a positive CATT-P titer ≥1:4 without parasitologic confirmation are considered as serologically suspected and are followed-up for one year. However, the efficiency of this strategy remains controversial, particularly in regions such as southern Sudan where follow-up is difficult because of long distances, lack of transport, and population movements. In such a setting, the determination of a CATT-P dilution titer above which individuals are likely to have the disease would allow the treatment of these infected individuals at an earlier stage and avoid the risk of losing these patients during follow-up. Moreover, with the treatment of a higher proportion of infected individuals who are the main reservoir of *T. b. gambiense*, control of the disease may be accelerated.

The few published studies addressing this question have led to controversial results. Of 86 serologically suspected individuals (CATT-P titer ≥1/4) followed in Angola, Simarro and others found that 52% of the individuals with a CATT end titer ≥1/16 were diagnosed with HAT during follow-up, but none of the individuals with lower CATT end-titers were diagnosed.[7] These investigators recommended treatment with pentamidine of all serologically suspected individuals with a CATT end titer ≥1/16 in this region. Conversely, Garcia and others found only one patient with HAT during a two-year longitudinal follow-up of 77 serologically suspected individuals in a low prevalence area of Côte d'Ivoire.[8] However, in the latter study, the CATT was performed on undiluted plasma and on blood with a maximum dilution of 1:4.

In this study, we retrospectively analyzed the initial CATT-P end-dilution titer and other biologic parameters as possible determinants of outcome in a large cohort of serologically suspected individuals followed-up for one year in Kajo-Keji County in southern Sudan.

MATERIALS AND METHODS

Between June 2000 and March 2002, all individuals presenting at the Sleeping Sickness Treatment Center (SSTC) located in Kiri in Kajo-Keji County were screened for HAT using a screening (passive) and diagnostic protocol described elsewhere.[9] Active screening was carried out with two mobile teams who systematically visited all villages since April 2001. All individuals found with a positive CATT-P titer of 1:4 during active screening were referred to the SSTC laboratory for further diagnostic procedures.

Serologically suspected individuals were defined as indi-

viduals with a positive CATT-P titer of 1/4 with no trypanosomes found in the cervical lymph nodes, blood (by the quantitative buffy coat technique or the hematocrit centrifugation technique), or CSF (after double centrifugation), and a CSF cell count $\leq 20/mm^3$. Using the same plasma sample, a serial two-fold dilution in CATT buffer up to a titer of 1:32 was performed for all serologically suspected individuals between June 2000 and March 2002, and the end-dilution titer was recorded. All serologically suspected individuals were then discharged from the SSTC laboratory and were requested to come back for control visits after 3, 6, and 12 months. The screening and diagnostic algorithm, as performed during the initial evaluation,[9] was repeated at each control visit. Individuals who did not attend follow-up were traced at home by locally trained Sleeping Sickness Assistants. Vehicle transport was not provided because of logistic constraints. The Ethical Review Board of Médecins Sans Frontières did not require formal ethical review because the analysis was performed on data routinely collected during the program.

Data collection. All sociodemographic and laboratory data, including the results of the CATT-P end-dilution titer and the laboratory results of the follow-up visits, were entered into *YoTryp*, a Microsoft Access-based software (Microsoft, Redmond, WA) specifically designed for the program. In March 2003, all data was transferred onto SPSS version 11.0 statistical software (SPSS, Inc., Chicago, IL) and analyzed.

Statistical analysis. Descriptive statistical analysis of sociodemographic characteristics and laboratory test results at screening of all serologically suspected individuals were performed using frequency tables. All subjects with at least one follow-up and the respective outcome were selected to calculate the incidence of HAT, using the number of HAT cases detected during follow-up as the numerator and the total person-year as the denominator. To assess whether selection bias occurred, sociodemographic characteristics and laboratory test results at screening of subjects with at least one follow-up were compared with those lost to follow-up, using cross-tabulations and chi-square tests. To study the association between laboratory test results at screening and the risk of developing HAT during follow-up, we calculated the incidence of HAT across four levels of CATT end-dilution serologic results (1:4, 1:8, 1:16, and 1:32) and three levels of CSF cell counts (0–5, 6–10, and 11–20) and the corresponding relative risks. Cox models were used to calculate relative risks adjusted for other significant factors and cumulative hazard over time of follow-up.

RESULTS

Between June 2000 and March 2002, 1,658 patients were diagnosed with HAT (740 with stage 1 disease and 918 with stage 2 disease) in the Kiri SSTC, while 2,355 individuals were defined as serologically suspected. The CATT-P end-dilution titer was determined in 2,274 of these 2,355 individuals (97%): the end-dilution titer was 1:4 in 1,086 (48%), 1:8 in 761 (33%), 1:16 in 264 (12%), and 1:32 in 163 (7%). The CATT-P end-dilution titer was not determined in 84 individuals (3%), who were therefore excluded from further analysis. The sociodemographic and laboratory characteristics of the 2,274 serologically suspected individuals included in the analysis are shown in Table 1.

The follow-up attendance of the serologically suspected individuals was low and decreased over time: 553 individuals (24%) were controlled at 3 months, 255 (11%) at 6 months, and 92 (4%) at 12 months. A total of 749 individuals (33%) were controlled at least once during follow-up. When compared with the 1,525 individuals who were lost to follow-up, these 749 individuals had a similar age, sex, CATT end-dilution titer distribution, and CSF cell count, but were statistically ($P < 0.05$) more likely to be displaced Sudanese, to come from the Bamurye camp, and to be detected by passive screening (Table 1).

Human African trypanosomiasis was diagnosed in 64 serologically suspected individuals during follow-up: 28 patients (44%) with first-stage illness and 36 patients (56%) with second-stage (or neurologic stage) illness. This represented 4% (64 of 1,658) of all patients diagnosed with HAT in the Kiri SSTC during the same period, 3% (64 of 2,274) of all individuals initially defined as serologically suspected, and 9% (64 of 749) of those who attended at least one follow-up.

Predictors of a diagnosis of HAT during follow-up of serologically suspected patients. Among the 749 serologically suspected individuals who were controlled at least once during follow-up, the sociodemographic characteristics and mode of screening of the 64 individuals diagnosed with HAT during follow-up were similar to those of the other 685 individuals. The proportion of serologically suspected individuals eventually diagnosed with HAT increased with higher initial CATT-P end-dilution titers (5%, 6.7%, 23.3%, and 19.6% for 1:4, 1:8, 1:16, and 1:32 titers, respectively) and with higher initial CSF cell counts (7.3%, 16.7%, and 34.8% for 0–5, 6–10, and 11–20 cells, respectively). Of the 64 HAT cases, 29 (45%) patients had an initial CATT end-titer of 1:16 or 1:32, while 8 (13%) patients (including 7 with second-stage illness) had an initial CSF cell counts of 11–20 cells.

The 749 serologically suspected individuals were followed for a total of 129,201 days. The overall risk/person-year of being diagnosed with HAT during follow-up was 18.1% and increased for higher CATT-P dilutions and higher CSF cell counts (Table 2). The risk was significantly higher in individuals with a CATT end-dilution titer $\geq$ 1/16 (Figure 1A) and in individuals with a CSF cell count of 11–20 (Figure 1B). This difference remained significant after adjustment for age, sex, mode of screening, CSF cell count, and CATT end-dilution titer. The combination of a CATT titer $\geq$ 1/16 and a CSF cell count of 11–20 had an additive effect on the cumulative risk, but this was found in only four individuals.

DISCUSSION

Because of the insufficient sensitivity of the current parasitologic field techniques and the limited specificity of the CATT-WB and CATT-P 1/4,[4] many African patients infected with *T. b. gambiense* go home undetected and untreated after completion of investigations. This situation is unsatisfactory not only for the infected individual, who will be more likely to be diagnosed at a later stage or simply die at home, but also for the community. Indeed, these individuals might maintain the human reservoir of the parasite and contribute to further transmission of the disease in the community. A possible approach is to follow-up and re-examine all serologically suspected individuals at regular intervals to detect and treat

TABLE 1

Sociodemographic characteristics and laboratory test results for African trypanomiasis at screening at 2,274 serologically suspect individuals, 749 individuals with at least one follow-up, and 1,525 individuals lost to follow-up in Kajo-Keji County, southern Sudan*

Characteristics	All serologically suspected no. (%)	≥1 Control visit during follow-up no. (%)	Lost to follow-up no. (%)	P†
Sex				0.97
Male	994 (44)	327 (44)	667 (44)	
Female	1,280 (56)	422 (56)	858 (56)	
Age (years)				0.21
0–4	82 (4)	20 (3)	62 (4)	
5–14	612 (27)	209 (28)	403 (26)	
>14	1,580 (70)	520 (69)	1,060 (70)	
Status				<0.001
Kajo-Keji resident	1,521 (67)	448 (60)	1,073 (70)	
Sudanese from displaced camp	686 (30)	279 (37)	407 (27)	
Non-Kajo-Keji residents	67 (3)	22 (3)	45 (3)	
Payam of residence				<0.001
Bamurye	570 (25)	260 (35)	310 (20)	
Kangapo 1	466 (20)	139 (19)	327 (21)	
Kangapo 2	921 (41)	220 (29)	701 (46)	
Lirye	111 (5)	54 (7)	57 (4)	
Livolo	105 (5)	43 (6)	62 (4)	
Lobonok/Ngepo	37 (2)	15 (2)	22 (2)	
Other county or Uganda	64 (3)	18 (2)	46 (3)	
Mode of screening				<0.001
Passive	1,360 (60)	539 (72)	821 (54)	
Active	914 (40)	210 (28)	704 (46)	
CATT end dilution				0.60
1:4	1,086 (48)	364 (49)	722 (47)	
1:8	761 (33)	253 (34)	508 (33)	
1:16	264 (12)	86 (11)	178 (12)	
1:32	163 (7)	46 (6)	117 (8)	
CSF cells‡				0.10
0–5	2,104 (93)	683 (92)	1,421 (94)	
6–10	105 (5)	35 (5)	70 (5)	
11–20	49 (2)	23 (3)	26 (2)	

* CATT = card agglutination test for trypanosomiasis; CSF = cerebrospinal fluid.
† by Pearson chi square test.
‡ Sixteen missing values.

those with a parasitologic confirmation during follow-up. Unfortunately, this strategy is very difficult to implement in the field and its effectiveness is low, as shown in our program in Kajo-Keji County in southern Sudan. Despite an effective team of 30 community-based sleeping sickness assistants, all well-motivated and regularly paid, the follow-up attendance of serologically suspected individuals and the overall yield of this strategy remained low.

Therefore, the early identification of serologically suspected individuals likely to be infected with *T. b. gambiense*

TABLE 2

Risk of developing human African trypanosomiasis according to CATT end dilution titer and CSF cell count at screening in 749 serologically suspected patients in Kajo-Keji county, southern Sudan*

Parameter	No. of cases of HAT diagnosed	No. serologically suspected	Total days of follow-up	Risk/100 person-years	Relative risk (95% CI)	Adjusted relative risk (95% CI)†
CATT end dilution titer						
1:4	18	364	63,524	10.4	1.0	1.0
1:8	17	253	44,868	13.8	1.3 (0.7–2.5)	1.1 (0.5–2.1)
1:16	20	86	13,859	52.7	5.1 (2.6–9.5)	4.4 (2.3–8.4)
1:32	9	46	6,950	47.3	4.6 (2.0–9.8)	4.1 (1.8–9.4)
CSF cell count‡						
0–5	50	685	118,086	15.5	1.0	1.0
6–10	6	36	5,884	37.3	2.4 (1.0–5.5)	2.0 (0.8–4.7)
11–20	8	23	3,836	76.2	4.9 (2.3–10.5)	4.4 (1.9–9.8)
Combination‡						
CATT 1:4–1:8 and cells 0–10	31	598	105,718	10.7	1.0	1.0
CATT 1:16–1:32 and cells 0–10	25	123	19,647	46.5	4.3 (2.5–7.3)	4.5 (2.6–7.8)
CATT 1:4–1:8 and cells 11–20	4	15	2,674	54.7	5.1 (1.8–14.9)	5.0 (1.7–14.2)
CATT 1:16–1:32 and cells 11–20	4	8	1,162	125.7	11.7 (4.1–33.2)	15.5 (4.9–49.7)

* CATT = card agglutination test for trypanosomiasis test; CSF = cerebrospinal fluid; CI = confidence interval.
† All relative risk adjusted for age, sex, mode of screening, CATT end dilution titer, and CSF cell count.
‡ Five missing values.

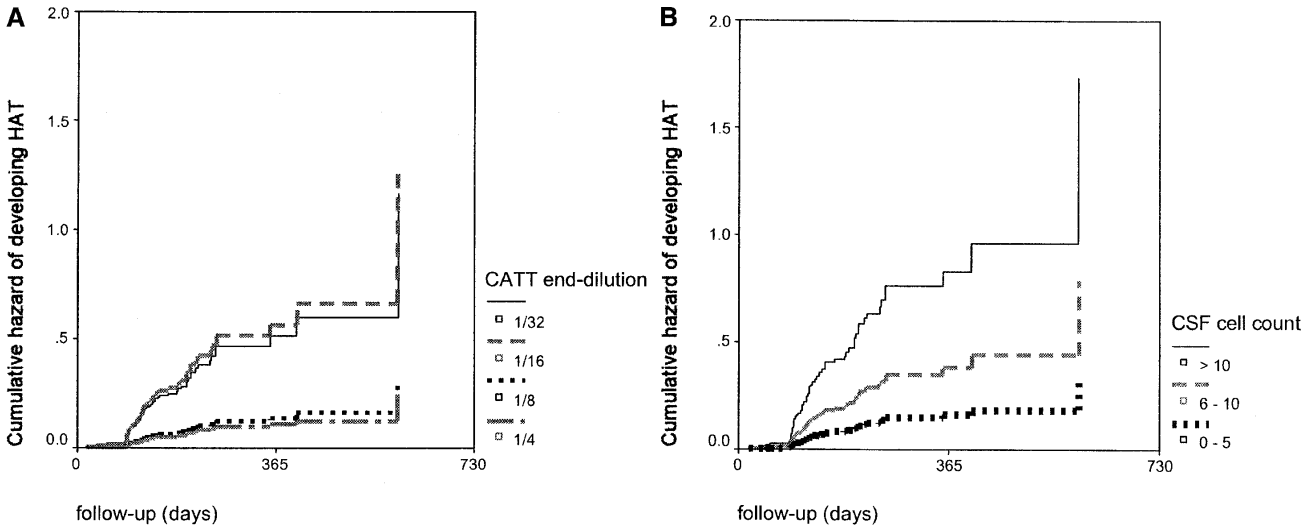

FIGURE 1. Cumulative risk of being diagnosed with human African trypanosomiasis (HAT) in relation to **A**, the card agglutination trypanosomiasis test (CATT)–plasma end-dilution titer and **B**, the cerebrospinal fluid (CSF) cell count during a one-year follow-up of 749 serologically suspected individuals in Kajo-Keji County in southern Sudan.

would be very useful. In this study, we analyzed the initial CATT end-dilution titers and the CSF cell counts of all serologically suspected individuals (n = 749) examined at least once during follow-up. We found that individuals with a CATT end-dilution titer of 1:16 or 1:32 had a higher risk of being diagnosed with HAT during follow-up (risk for 100 person-years = 53% and 47%, respectively), a significantly higher risk (adjusted relative risk [RR] = 4.4 and 4.1, respectively) than individuals with a CATT end-dilution titer of 1:4 (risk for 100 person-years = 10%). Similar findings have been reported by Simarro and others in a smaller study in Angola.[7]

If the 132 individuals with a CATT titer ≥1/16 had been treated after initial evaluation, 29 (22%) individuals subsequently diagnosed with HAT would have been treated and cured (provided that treatment was 100% efficient). However, 103 (78%) individuals would have been treated in excess. The PPV of a CATT titer ≥1/16, like any other diagnostic test, depends on the prevalence of the disease in the tested population. The PPV was moderate (22%) in Kajo-Keji, where the mean prevalence of HAT found during active screening sessions was 1%, but was higher (52%) in the study by Simarro and others in Angola, in which the prevalence of HAT in the studied focus was 1.9%.[7] This concept is crucial for policy makers and it would be extremely useful in the future to gather and analyze all published and unpublished data on that issue to determine a prevalence cut-off above which treatment of individuals with a CATT titer ≥1/16 is worthwhile. The decision should also depend on other factors such as the availability and cost of treatment, the capacity to perform repeated active screening sessions (potentially limited by geographic, safety, time, and/or transport constraints), and the feasibility and expected effectiveness of a follow-up strategy.

As suggested by Simarro and others, pentamidine is the only therapeutic option for treating individuals with a CATT titer ≥1/16.[7] Lumbar puncture should be performed in these individuals to rule out second-stage illness (presence of trypanosomes and/or >20 cells in the CSF). Despite the painful daily intramuscular injections and the possible occurrence of side effects such as hypoglycemia and hypotension, pentamidine therapy is relatively short (seven days) and very safe. In Kajo-Keji, only 2 of more than 2,000 patients treated with pentamidine died during treatment, and this was due to non–drug-related causes. Pentamidine would not only be very efficient in treating undiagnosed first-stage patients (CSF cell counts = 0–5 cells), who would represent the highest proportion of individuals (92% in Kajo-Keji), but would also have some degree of efficiency in undiagnosed early second-stage patients (CSF cell counts = 6–20 cells).[10,11] Ongoing separate studies are currently assessing the efficacy of a shortened schedule (three days) of pentamidine and an oral drug, DB 289, for the treatment of first-stage HAT. The availability of an oral treatment or, to a lesser extent, a shorter parenteral treatment would greatly facilitate the treatment of both first-stage and serologically suspected patients in the field.

An initial CSF cell count of 11–20 was also found to be a strong risk factor for serologically suspected individuals diagnosed with HAT during follow-up with a risk for 100 person-years of 76% and an adjusted RR of 4.4 compared with individuals with 0–5 cells in the CSF. However, this risk factor was only found in 8 (13%) individuals diagnosed with HAT during follow-up, all but one with second-stage disease. If one considers the high toxicity of melarsoprol and the complicated schedule of eflornithine (14 days of 4 daily slow infusions), the only two drugs currently registered for the treatment of second-stage illness, their use should be restricted to confirmed cases.

Our study had one main potential limitation. The extrapolation of our results to the whole population of serologically suspected individuals could be limited by the low attendance rate obtained during follow-up. Individuals with an unknown outcome (lost to follow-up) had a significantly different status, location of residence, and mode of screening, but had comparable CATT-P end-dilution titers and CSF cell counts, the only factors found to be associated with a higher risk of HAT. The attendance of follow-up visits was therefore influenced more by factors such as distance, mobility and motivation. Therefore, we believe that it is reasonable to extrapolate

our results to the whole population of serologically suspected individuals diagnosed during the study period in Kajo-Keji.

In conclusion, we showed that the follow-up of serologically suspected individuals had a low effectiveness and yield in a field setting such as southern Sudan. We identified a CATT-P titer $\geq$1/16 as a useful predictor of HAT in this population. We suggest that treatment of these individuals with pentamidine is worthwhile, both on an individual and public health basis, if the prevalence of HAT in the population investigated is sufficiently high. Which cut-off of prevalence to choose remains an open question. It should probably not be less than 1% and the decision should be tempered by other factors such as distance to the treatment center and means of transportation. Based on these results, all serologically suspected individuals with a CATT-P titer $\geq$1/16 diagnosed in Kajo-Keji County during passive screening or first-round active screening (the settings where the highest prevalence of HAT is found) since March 2002 have received treatment with pentamidine.

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