MAJOR ARTICLE

How to Shorten Patient Follow-Up after Treatment for *Trypanosoma brucei gambiense* Sleeping Sickness

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Background. Clinical management of human African trypanosomiasis requires patient follow-up of 2 years' duration. At each follow-up visit, cerebrospinal fluid (CSF) is examined for trypanosomes and white blood cells (WBCs). Shortening follow-up would improve patient comfort and facilitate control of human African trypanosomiasis.

Methods. A prospective study of 360 patients was performed in the Democratic Republic of the Congo. The primary outcomes of the study were cure, relapse, and death. The WBC count, immunoglobulin M level, and specific antibody levels in CSF samples were evaluated to detect treatment failure. The sensitivity and specificity of shortened follow-up algorithms were calculated.

Results. The treatment failure rate was 37%. Trypanosomes, a WBC count of $\geqslant$100 cells/μL, and a LATEX/immunoglobulin M titer of $\geqslant$1:16 in CSF before treatment were risk factors for treatment failure, whereas human immunodeficiency virus infection status was not a risk factor. The following algorithm, which had 97.8% specificity and 94.4% sensitivity, is proposed for shortening the duration of follow-up: at 6 months, patients with trypanosomes or a WBC count of $\geqslant$50 cells/μL in CSF are considered to have treatment failure, whereas patients with a CSF WBC count of $\leqslant$5 cells/μL are considered to be cured and can discontinue follow-up. At 12 months, the remaining patients (those with a WBC count of 6–49 cells/μL) need a test of cure, based on trypanosome presence and WBC count, applying a cutoff value of 20 cells/μL.

Conclusion. Combining criteria for failure and cure allows follow-up of patients with second-stage human African trypanosomiasis to be shortened to a maximum duration of 12 months.

Human African trypanosomiasis (HAT) is endemic in sub-Saharan Africa. *Trypanosoma brucei gambiense* causes chronic HAT in West and Central Africa. In 2006, a total of 11,382 cases were reported [1]. There are 2 disease stages: first stage (characterized by the presence of trypanosomes in peripheral tissues and organs) and second stage (characterized by the presence of trypanosomes in the brain). Treatment is stage dependent, and untreated HAT is fatal.

Clinical management of HAT requires patient follow-up of 2 years' duration, with visits occurring at 3, 6, 12, 18, and 24 months after treatment [2]. At each follow-up visit, lymph, blood, and cerebrospinal fluid (CSF) samples are examined for trypanosomes, and CSF white blood cell (WBC) counts are determined. Compliance of patients with HAT with scheduled follow-up decreases with time, from 65%–85% at 12 months to 25%–70% at 24 months, whereas 40%–90% of relapses occur within 12 months and 70%–90% occur within 18 months [3]. For clinical research, 18 months of follow-up were recommended [3].

Patients with second-stage disease who had a CSF WBC count of $\leqslant$5 cells/μL at 6 months are at low risk of relapse [4], and defining relapse as "trypanosomes present and/or $\geqslant$50 WBC/μL CSF" allowed timely and

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accurate detection of relapse at any time point during follow-up [5]. Using detection of immunoglobulin M (IgM) and trypanosome-specific antibodies, in combination with the CSF WBC count and presence of trypanosomes, might also allow for a shorter follow-up [4] and, thus, improve patient comfort and facilitate HAT control.

This study investigates whether and how follow-up of patients with HAT can be shortened by applying novel biological criteria under field conditions. We report the treatment outcomes of a prospectively recruited cohort of patients, evaluate risk factors and biomarkers for treatment failure, and validate criteria for shorter follow-up periods.

METHODS

Study design. A longitudinal study was performed to evaluate biomarkers for monitoring the clinical outcome of patients treated for HAT. Primary outcomes of the study were relapse, cure, and death. The secondary outcome was the time of treatment failure. Ethics committees of The Ministry of Health, Democratic Republic of the Congo, and the University of Antwerp, Belgium, provided ethics clearance.

Study site. The study was conducted at Dipumba Hospital, Mbuji Mayi, East Kasai Province, Democratic Republic of the Congo. In 2005, the national control program (Programme National de Lutte contre la Trypansomiase Humaine Africaine [PNLTHA]) reported that the prevalence of HAT in this province was 0.84% among 327,498 persons screened. Treatment failure rates for patients treated with melarsoprol in Dipumba Hospital were close to 50% in 2003–2004 [6].

Patients. The study was performed between May 2005 and May 2008, with patient recruitment continuing until February 2006. Inclusion criteria were the presence of trypanosomes in lymph, blood, or CSF, regardless of disease stage; age $\geqslant$12 years; and residence within a 100-km perimeter around Mbuji-Mayi. Exclusion criteria were pregnancy, no guarantee of follow-up, moribund status, hemorrhagic CSF, and concurrent serious illness (tuberculosis or bacterial or cryptococcal meningitis). Patients who had never been treated previously for HAT (ie, treatment-naive patients) were classified as "primary cases," and those who presented with relapse at enrollment were classified as "retreatment cases." Informed consent was received from the patients or their guardian before enrollment.

Diagnosis. Each patient underwent a clinical examination. Whole blood was tested for specific antibodies, by use of the card agglutination test for trypanosomiasis (CATT) [7]. CATT-positive individuals and those showing suggestive clinical signs were examined for the presence of trypanosomes in lymph or in blood via capillary tube centrifugation or a mini-anion exchange centrifugation technique [8, 9]. A CSF WBC count was determined using disposable counting chambers (Uriglass; Menarini), and a search for trypanosomes was conducted using modified single centrifugation [10]. Retreatment cases were enrolled on the basis of a trypanosome-positive CSF sample. Disease staging was as follows: first stage was defined by a WBC count of 0–5 cells/μL and no trypanosomes present in CSF, and second stage was defined by a WBC count of >5 cells/μL and/or presence of trypanosomes in CSF. Trypanosome-specific antibodies and the total IgM level in CSF were measured using LATEX/*T. b. gambiense* and LATEX/IgM [11, 12]. All tests were repeated 24 h after the last drug administration and at each follow-up assessment. Demographic and clinical characteristics were recorded on a case report form and were entered into an Epi-Info database (version 3.4.3; Centers for Disease Control and Prevention and World Health Organization [WHO]). Human immunodeficiency virus (HIV) infection status was determined with the Vironostika HIV Uni-Form II Ag/Ab (bioMérieux), followed by the INNO-LIA HIV I/II Score (Innogenetics), if the finding was reactive. The INNOTEST HIV Antigen mAb enzyme-linked immunosorbent assay (Innogenetics) was used to detect early seroconversions. HIV polymerase chain reaction analysis of peripheral blood mononuclear cells was performed to confirm HIV infection [13, 14]. HIV-seropositive patients who consented to be informed about their status were referred to the HIV counseling service at Dipumba Hospital.

Treatment. Patients received free treatment and food during hospitalization. Pretreatment consisted of mebendazole (100 mg twice daily for 3 days) and a single dose of combination treatment with 500 mg of sulfadoxine and 25 mg of pyrimethamine (1 tablet per 20 kg of body weight). HAT treatment (Table 1) was given in accordance with PNLTHA guidelines.

Patient monitoring after treatment. Treatment outcome was assessed 1 day (ie, at the end of treatment) and at 3, 6, 12, 18, and 24 months after treatment. During follow-up, patients were classified as having the following outcomes, in accordance with WHO recommendations [3]: death, relapse, probable relapse, favorable evolution, or uncertain evolution (with the following adaptations). Probable relapse was defined by no trypanosomes detected and either a CSF WBC count that increased by >30 cells/μL, compared with the lowest previous WBC count, or neurologic signs not resulting from a condition other than HAT and requiring rescue treatment in the opinion of the physician in charge. Favorable evolution was defined as follows: for stage 1, a WBC count of $\leqslant$5 cells/μL and no trypanosomes detected; for stage 2, either no trypanosomes detected and a WBC count of $\leqslant$20 cells/μL or no trypanosomes detected and a WBC count that had not increased by >10 cells/μL, compared with the lowest previous value, and without clinical deterioration. Uncertain evolution was defined by no trypanosomes detected and failure to be classified as belonging to any other category. For test-of-cure

Table 1. Treatment Regimens Administered to Patients with Human African Trypanosomiasis (HAT) Who Were Included in a Study Performed at Dipumba Hospital, Democratic Republic of the Congo

Drug(s) and dosage(s)	Case status
Pentamidine, 4 mg/kg/day IM for 8 days	Primary case, first stage
Melarsoprol,[a] 2.2 mg/kg/day IV for 10 days	Primary case, second stage
	Retreatment case after treatment of first-stage disease with pentamidine
Melarsoprol, 1.8 mg/kg/day IV for 10 days, PLUS nifurtimox; 15 mg/kg/day PO for 14 days	Retreatment case with no intolerance to melarsoprol noted during previous treatment
Eflornithine, 4 × 100 mg/kg/day IV every 6 h for 14 days	Primary case, second stage
	Primary case, second stage, with intolerance to melarsoprol developing within 8 days of treatment
	Retreatment case with intolerance to melarsoprol having developed during previous treatment
	Retreatment case treated with melarsoprol-nifurtimox combination therapy that was prematurely terminated when nifurtimox was no longer available
	Retreatment case previously treated with melarsoprol-nifurtimox combination therapy
Eflornithine, 4 × 100 mg/kg/day IV every 6 h for 14 days, PLUS melarsoprol, 3.6 mg/kg/day IV; 3 series of 3 daily injections were administered at 7-day intervals	Retreatment case, with no drug intolerance noted during previous treatments with melarsoprol, eflornithine, or a combination of both
Eflornithine, 4 × 100 mg/kg/day IV every 6 h for 14 days, PLUS nifurtimox, 15 mg/kg/day PO for 14 days	Retreatment case; patients had previously received treatment with eflornithine or had developed intolerance to previous treatment with regimens containing melarsoprol

NOTE. IM, intramuscular; IV, intravenous; PO, by mouth.

[a] At the first sign of reaction, treatment was interrupted; treatment received until day 8 was considered to be complete.

(ToC) assessment at 24 months or later, WHO recommendations were followed [3].

Data analysis. Tolerance windows of 2–4, 5–9, 10–16, 17–21, and ⩾22 months were defined around the scheduled follow-up at 3, 6, 12, 18, and 24 months [3]. Patients attaining a final treatment outcome (nonresponder status, relapse, probable relapse, or death) before the ToC assessment at 24 months were excluded from data analysis at subsequent time points. EpiInfo software (version 3.4.3) and Stata software (version 10; Stata) were used for data analysis, with statistical significance denoted by $P = .05$. Comparisons between treatment regimens or algorithms were done with Kruskal-Wallis, Pearson's $\chi 2$, or Fisher's exact tests.

Patients classified as experiencing relapse, probable relapse, failure to respond, or death possibly related to HAT during follow-up were considered to have treatment failure. To calculate the risk factors for treatment failure, to determine marker accuracy, and to evaluate algorithms for follow-up, only data for patients with second-stage disease were analyzed, excluding patients whose deaths were not associated with HAT and those with unknown treatment outcomes. To assess the risk of treatment failure, adjusted odds ratios were estimated based on logistic regression models.

The accuracy of continuous markers (the CSF WBC count and the LATEX/IgM titer) in the determination of treatment failure was assessed by constructing the receiver operator characteristic (ROC) curve and calculating the area under the curve (AUC). When the AUC value was >0.80, the Youden index was determined for the whole range of cutoff values, and the cutoff value with the maximal Youden index was retained. The sensitivity and specificity of some additional cutoff values for the WBC count (⩽5 cells/μL, ⩽20 cells/μL, and ⩾50 cells/μL) were calculated [3–5]. For the binary marker (LATEX/*T. b. gambiense*), accuracy was estimated by calculating sensitivity, specificity, and Youden index.

Several clinical algorithms for detecting failure in association with a shorter follow-up were constructed and evaluated using the cohort data. The best algorithms were validated using an independent data set obtained in a clinical trial conducted between 1998 and 2001 in Bwamanda, Democratic Republic of the Congo [4, 5, 15].

RESULTS

Study population. A total of 360 patients with HAT were enrolled (Figure 1). Their characteristics at baseline are shown in Table 2. More male patients than female patients were enrolled in the groups for the treatment of second-stage disease ($P < .001$), but the sex ratio was not different from 1 in the group with first-stage disease. Two hundred forty-two patients were primary cases, and 118 were retreatment cases; 41 had first-stage disease, and 319 had second-stage disease. Trypan-

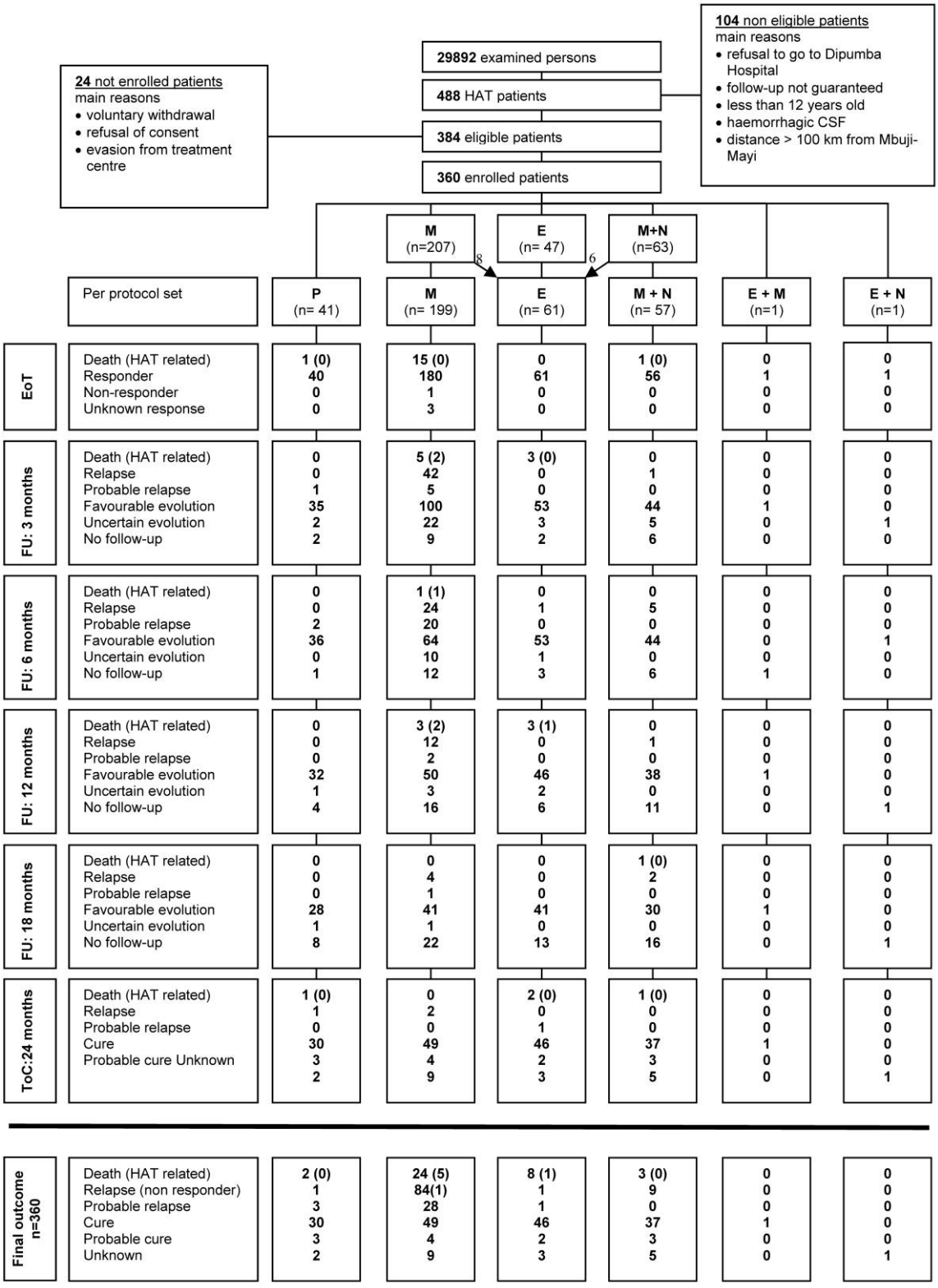

Figure 1. Overview of study profile. CSF, cerebrospinal fluid; E, eflornithine; E-M, eflornithine-melarsoprol combination therapy; E-N, eflornithine-nifurtimox combination therapy; EoT, end-of-treatment assessment; interim FU, follow-up assessments performed at 3, 6, 12 and 18 months; HAT, human African trypanosomiasis; M, melarsoprol; M-N, melarsoprol-nifurtimox combination therapy; N, nifurtimox; P, pentamidine; ToC, test-of-cure assessment performed 24 months after treatment.

Table 2. Baseline Characteristics of 360 Patients Enrolled in the Study, by Treatment Received

Characteristic	All patients (n = 360)	P (n = 41)	M (n = 199)	E (n = 61)	M-N (n = 57)	M-E (n = 1)	E-N (n = 1)
Demographic							
Ratio of male patients to female patients, no.	250:110	18:23	143:56	44:17	44:13	1:0	0:1
Age, mean ± SD, years	34 ± 12	35 ± 13	34 ± 13	34 ± 11	34 ± 12	32	28
Weight, mean ± SD, kg	56 ± 10	53 ± 9	55 ± 10	58 ± 9	59 ± 11	60	63
Height, mean ± SD, cm	168 ± 10	163 ± 9	167 ± 10	170 ± 9	169 ± 10	167	170
Active:passive case detection ratio[a]	61:299	…	…	…	…	…	…
Primary:retreatment case ratio[b]	242:118	41:0	192:7	9:52	0:57	0:1	0:1
Trypanosomes detected[c]							
In lymph	166/227	30/39	133/182	3/6	0/0	0/0	0/0
In blood							
By CTC	24/86	6/11	18/65	0/8	0/0	0/0	0/0
By mAECT	17/59	5/5	8/45	4/8	0/0	0/0	0/0
In CSF							
In cell-counting chamber	152/360	0/41	96/199	30/61	25/57	1/1	0/1
By modified single centrifugation	142/208	0/41	78/103	31/31	32/32	0/0	1/1
CSF biological							
WBC count, mean ± SD, cells/μL	267 ± 295	2 ± 1	329 ± 341	276 ± 216	233 ± 179	214	208
LATEX/IgM titer, mean ± SD	70 ± 100	1 ± 3	75 ± 112	84 ± 84	92 ± 90	16	64
LATEX/*T. b. gambiense* result, n/N[d]	259/357	3/41	150/197	56/61	48/56	1/1	1/1
HIV infection status, n/N[d]	11/360	0/41	9/199	1/61	1/57	0/1	0/1

NOTE. CSF, cerebrospinal fluid; CTC, capillary tube centrifugation; E, eflornithine; E-N, eflornithine-nifurtimox combination therapy; HIV, human immunodeficiency virus; IgM, immunoglobulin M; M, melarsoprol; mAECT, mini-anion exchange centrifugation technique; M-E, melarsoprol-eflornithine combination therapy; M-N, melarsoprol-nifurtimox combination therapy; N, nifurtimox; P, pentamidine; SD, standard deviation; *T. b. gambiense, Trypanosoma brucei gambiense;* WBC, white blood cell.

[a] Data are the no. of patients with active case detection/no. of patients with passive case detection.

[b] Data are the ratio of patients considered to be "primary cases" (ie, patients who had never been treated previously for human African trypanosomiasis [treatment-naive patients]) to patients considered to be "retreatment cases" (ie, patients who presented with relapse at enrollment).

[c] Data are the no. of patients with trypanosomes detected/no. of patients assessed.

[d] Data are the no. of patients with a positive result/no. of patients assessed.

osomes were detected in the CSF of 294 patients. Most patients with second-stage disease had high CSF WBC counts (mean ± SD, 301 ± 297 cells/μL) and positive results of LATEX/*T. b. gambiense* performed on CSF samples. HIV prevalence was 3.1% (11 of 360 patients; 95% confidence interval [CI], 1.6%–5.6%).

Treatment outcomes. The patients (Figure 1) were treated as follows: 41 received pentamidine; 207 started receiving melarsoprol (M) monotherapy, but 8 developed intolerance to M before day 8 and had treatment switched to eflornithine (E); 63 received combination therapy with M and nifurtimox (M-N), but 6 had treatment switched to E when nifurtimox (N) was no longer available; 1 received combination therapy with M and E; and 1 received combination therapy with N and E. A total of 61 patients received E monotherapy. A total of 250 patients completed prescribed treatment without interruptions, and 66 patients (28 who were receiving E, 15 who were receiving M, and 23 who were receiving M-N) experienced minor temporary treatment interruptions. Forty-four patients (1 who was receiving E, 36 who were receiving M, and 7 who were receiving M-N) had treatment terminated before the total prescribed dosage was administered; however, the required minimum dosage [3] was reached for all.

Adherence to follow-up examinations (including lumbar puncture) was, respectively, 94.4% (323 of 342 patients) at 3 months, 91.9% (262 of 285 patients) at 6 months, 83.6% (194 of 232 patients) at 12 months, 71.6% (151 of 211 patients) at 18 months, and 84.2% (171 of 203 patients) at 24 months. It was not significantly different between treatment groups.

The overall cure rate was 48.6% (175 of 360 patients), and the case-fatality rate was 10.3% (37 of 360 patients). Seventeen patients died during treatment: 5 (4 receiving M and 1 receiving M-N) died of advanced HAT, 10 died of M toxicity, 1 (who was receiving M) died of septicemia; and 1 (who was receiving P) died of an unknown cause. During follow-up, 20 deaths occurred, 6 of which were due to HAT (5 patients who died were receiving M, and 1 patient was receiving E); 1, to E toxicity; 1, to hepatorenal failure (the patient was receiving M); 1, to status epilepticus (the patient was receiving E); and 11, to unknown causes (5 patients were receiving E, 3 were receiving M,

2 were receiving M+N, and 1 was receiving P). At the end of treatment, 1 patient with trypanosomes in CSF was classified as a nonresponder. The overall treatment failure rate was 36.9% (133 of 360 patients): 1 patient had no response, 94 experienced relapse, 32 had a probable relapse, and 6 died of HAT-related causes during follow-up. In the remainder of this article, those events will be jointly classified as denoting "failure." The highest failure rate, 58.8% (117 of 199 patients), was observed in the M group, and the lowest failure rate (0%–5%) was observed for patients receiving regimens containing E. One relapse and 3 probable relapses occurred in the P group (9.8% [4 of 41 patients]). Of all failures, 38.3% (51 of 133 patients) were detected within 3 months, 78.2% (104 of 133 patients) within 6 months, 91.7% (122 of 133 patients) within 12 months, and 97.0% (129 of 133 patients) within 18 months.

Characteristics at baseline as risk factors for treatment failure in patients with second-stage disease. A WBC count of $\geqslant$100 cells/μL at baseline ($P = .008$), presence of trypanosomes in CSF ($P = .005$) at baseline, and a LATEX/IgM titer of $\geqslant$1:16 at baseline ($P < .001$) were significantly associated with higher failure rates after adjustment for drug regimen in patients with second-stage disease. Sex, age, trypanosome-specific antibodies (LATEX/*T. b. gambiense*), early treatment termination, and HIV infection status were not (Table 3). In the final logistic regression model, only the drug regimen and the LATEX/IgM titer at baseline were retained as independent predictors of failure.

Posttreatment evolution of CSF parameters in patients with second-stage disease. In patients considered to be cured, the median CSF WBC count decreased from 213 cells/μL to 36 cells/μL at the end of treatment and steadily decreased during follow-up (Figure 2), whereas among patients considered to have experienced treatment failure, it decreased from 259 cells/μL to 59 cells/μL at the end of treatment, increased to 101 cells/μL at 6 months, and subsequently decreased to 37 cells/μL at 24 months. In cured patients, median LATEX/IgM titers were 1:64 before treatment and decreased to 1:2 at 18 and 24 months, whereas in patients with treatment failure, they decreased until 3 months, stabilized at 1:16 between 3 and 12 months, and decreased further thereafter. The proportion of patients with a positive LATEX/*T. b. gambiense* result decreased from 82% before treatment to 14% at 24 months in patients considered to be cured, whereas in patients with treatment failure, it decreased from 80% before treatment to 52% at 3 months and increased to 56%–68% between 6 and 18 months.

Accuracy of criteria for detection of treatment failure in patients with second-stage disease. For the CSF WBC count, the AUC was >0.80 from months 3 to 24 (table 4). The Youden index was highest at 12 and 18 months, with 87%–89% sensitivity and 100% specificity at cutoff values of 23 and 29 cells/μL, respectively (table 4), although a cutoff value of >20 cells/μL had similar high sensitivities and specificities. A cutoff WBC count of >5 cells/μL was 89%–96% sensitive, and a cutoff WBC count of $\geqslant$50 cells/μL was 95%–100% specific.

For the CSF LATEX/IgM titer, the AUC was >0.80 at 12 months only (0.84; 95% CI, 0.72–0.96), with a cutoff value of $\geqslant$1:16. Sensitivity was 69%, and specificity was 97%.

For trypanosome-specific CSF antibodies, the maximum Youden index was 0.43 at 12 months. Sensitivity was 65%, and specificity was 78%.

Evaluation of algorithms for shortening the duration of follow-up. On the basis of the information presented above, we constructed several algorithms with variable durations of follow-up and used a composite definition to discriminate between cure and treatment failure at each follow-up visit. According to this definition, patients with a WBC count of $\leqslant$5 cells/μL at any follow-up visit are considered to be "cured" and do not require further follow-up, whereas those with a count of $\geqslant$50 cells/μL are considered to have "failure" and should receive rescue treatment. Trypanosome-negative patients with a WBC count of 6–49 cells/μL are considered to have "uncertain evolution" and should continue follow-up. At the final ToC assessment, patients with trypanosomes present or >20 WBCs/μL are considered to have "treatment failure." Combining these follow-up and ToC criteria, 4 algorithms were formulated, and their accuracy was checked against our study data (Figure 3).

In algorithm A, follow-up lasted for 24 months. Of patients with second-stage disease with known treatment outcome, 85 were considered to be cured at 6 months, 40 at 12 months, 7 at 18 months, and 10 at 24 months (for a total of 142 patients, including 4 whose outcomes were wrongly classified); 56 patients are considered to have treatment failure at 6 months, 10 at 12 months, and 6 at 18 months (for a total of 72 patients, including 2 whose outcomes were wrongly classified). Over 24 months, specificity is 98.6% (138 of 140 patients; 95% CI, 95%–100%) and sensitivity is 94.6% (70 of 74 patients; 95% CI, 87%–99%).

In algorithm B, follow-up lasted for 18 months. Eighty-five patients were considered to be cured at 6 months, 40 at 12 months, and 9 at 18 months (for a total of 134 patients, including 4 whose outcomes were wrongly classified); 56 patients were considered to have treatment failure at 6 months, 10 at 12 months, and 6 at 18 months (for a total of 72 patients, including 2 whose outcomes were wrongly classified). Specificity was 98.5% (130 of 132 patients; 95% CI, 95%–100%), and sensitivity was 94.6% (70 of 74 patients; 95% CI, 87%–99%).

In algorithm C, follow-up lasted for 12 months; 85 patients were considered to be cured at 6 months and 50 at 12 months (for a total of 135 patients, including 4 whose outcomes were wrongly classified); 56 patients were considered to have treatment failure at 6 months and 15 patients at 12 months (for a

Table 3. Risk Factors at Baseline for Treatment Failure in 272 Patients with Second-Stage Gambiense Sleeping Sickness

	Patients with treatment failure		Unadjusted		Adjusted for treatment		Multivariate model[a]	
Risk factor	*n/N*	%	OR	*P*	OR	*P*	OR	*P*
Treatment				<.001[b]				<.001[b]
E	3/51	6	1				1	
M	117/171	69	35.62				46.1	
M-E	0/1	0	–				–	
M-N	9/49	18	3.60				1.56	
Sex				.392		.655		
Female	39/75	52	1		1			
Male	91/197	46	0.79		0.86			
Age, years				.894		.726		
<25	34/69	49	1		1			
25–39	54/117	46	0.88		1.18			
⩾40	42/86	49	0.98		0.90			
WBC count, cells/μL				.091		.008[b]		
<100	22/63	35	1		1			
100–199	32/58	55	2.29		4.22			
200–399	38/79	48	1.73		3.21			
⩾400	38/71	54	2.15		1.95			
Trypanosomes in CSF				.467		.005[b]		
No	8/20	40	1		1			
Yes	122/252	48	1.41		1.52			
LATEX/IgM titer				.019[b]		<.001[b]		<.001[b]
<1:16	7/26	27	1		1		1	
⩾1:16	122/241	51	2.78		6.97		6.97	
LATEX/*T. b. gambiense* result				.611		.203		
Negative	26/51	51	1		1			
Positive	103/219	47	0.85		0.83			
Early treatment termination				.108		.648		
No	112/243	46	1		1			
Yes	18/29	62	1.91		1.22			
HIV infection status				.140		.334		
Negative	124/264	47	1		1			
Positive	6/8	75	3.39		2.42			

NOTE. Forty-seven patients were excluded because of missing data. CSF, cerebrospinal fluid; E, eflornithine; E-N, eflornithine-nifurtimox combination therapy; HIV, human immunodeficiency virus; IgM, immunoglobulin M; M, melarsoprol; M-E, melarsoprol-eflornithine combination therapy; M-N, melarsoprol-nifurtimox combination therapy; N, nifurtimox; OR, odds ratio; *T. b. gambiense; Trypanosoma brucei gambiense;* WBC, white blood cell.

[a] Multivariate model obtained by stepwise model selection using all factors significant in treatment-adjusted analysis.

[b] Statistically significant difference ($P<.05$).

total of 71 patients, including 3 whose outcomes were wrongly classified). Over 12 months, specificity was 97.8% (131 of 134 patients; 95% CI, 94%–100%), and sensitivity was 94.4% (68 of 72 patients; 95% CI, 86%–98%).

In algorithm D, the ToC criterion was applied at 6 months; 136 patients were considered to be cured (including 13 patients whose outcomes were wrongly classified); 77 patients were considered to have treatment failure (including 13 patients whose outcomes were wrongly classified). Specificity was 90.4% (123 of 136 patients; 95% CI, 84%–95%), and sensitivity was 83.1% (64 of 77 patients; 95% CI, 73%–91%).

To validate our findings, we applied these algorithms to a set of data for patients with second-stage and intermediate HAT who were treated with M, N, and a combination of both drugs (M-N) in another clinical trial [4, 5, 15]. The specificity and sensitivity for algorithm B were 98.9% (173 of 175 patients; 95% CI, 96%–100%) and 84.2% (32 of 38 patients; 95% CI, 69%–94%), respectively, and those for algorithm C were 98.8%

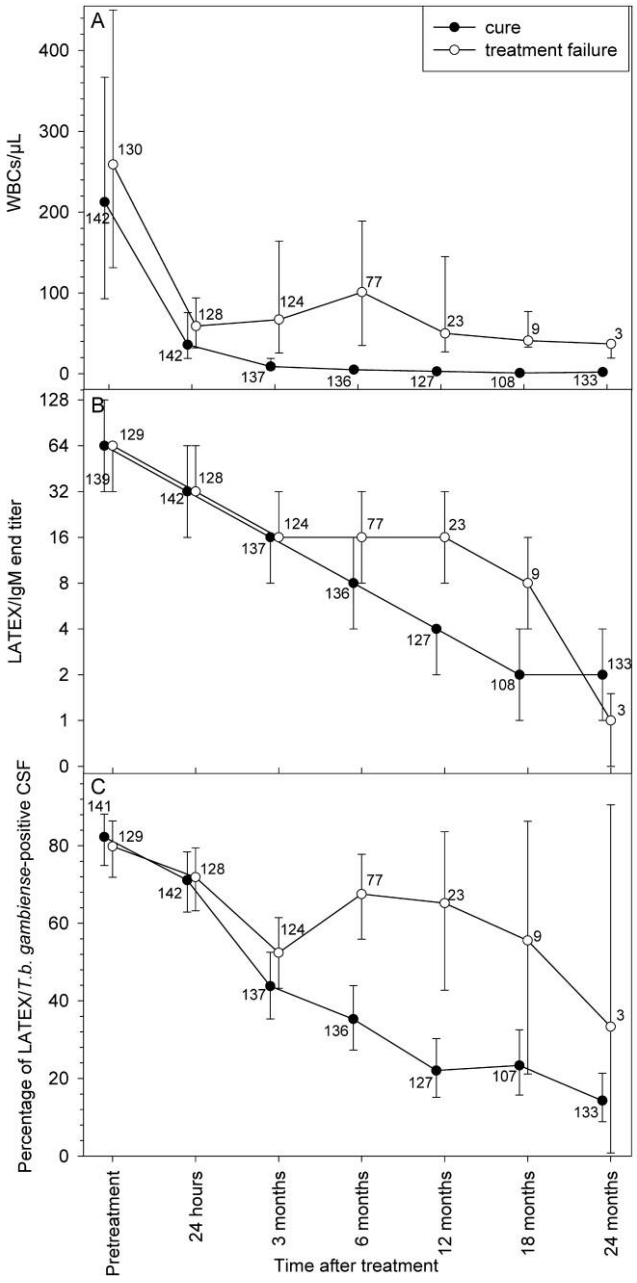

Figure 2. Evolution of the cerebrospinal fluid (CSF) white blood cell (WBC) count (expressed as the median and interquartile range) *(top),* LATEX/immunoglobulin M titer (expressed as the median and interquartile range) *(middle),* and LATEX/*Trypanosoma brucei gambiense* positivity (expressed as the percentage of CSF-positive patients and 95% confidence interval) *(bottom)* after treatment of human African trypanosomiasis in patients with second-stage disease. The no. of patients is indicated next to the data point.

(167 of 169 patients; 95% CI, 96%–100%) and 79.4% (27 of 34 patients; 95% CI, 62%–91%), respectively. For algorithm A, specificity was 99.5% (207 of 208 patients; 95% CI, 97%–100%), and sensitivity was 85.4% (35 of 41 patients; 95% CI, 71%–94%).

DISCUSSION

The present study was conducted to assess how the recommended 24 months of posttreatment follow-up for patients with HAT could be shortened using field-applicable criteria. The overall relapse rate in the cohort was 37%, but it was 59% after treatment with M for 10 days, a rate that is much higher than that reported elsewhere [16]. High rates of treatment failure after M monotherapy have also been observed in the Equateur province, Democratic Republic of the Congo [17]; in Uganda [18]; and in Angola [19]. It remains unclear whether these high rates are associated with the parasite or with patient characteristics. In 2006, PNLTHA replaced the 10-day M regimen with 14 days of E or M-N combination therapy, which subsequently reduced the treatment failure rate.

The >71% rate of adherence to follow-up in this cohort study was excellent and was achieved thanks to active tracing of patients who defaulted from follow-up. A lack of means of transportation was the main obstacle to completion of follow-up. Financial constraints constituted not only a barrier for early diagnosis and treatment [20] but also for follow-up compliance.

Within 6 months after treatment, 78% of all relapses were detected, a rate that is considerably higher than that noted in other studies with active follow-up: 17%, (in the 2004 study by Priotto detailed in [3]), 33% [16], and 50% [21]. The early relapse rate might be associated with the low efficacy of M, but it may also result from active tracing of patients defaulting from follow-up combined with the use of a very sensitive trypanosome detection technique, the modified single centrifugation of CSF. Also, the choice of criteria for relapse and probable relapse influences the sensitivity for and delay in detection of treatment failure [5].

HIV prevalence among the patients was 3.1%. This prevalence corresponded to the rates reported for the adult population in the study region [22].

After adjustment for the drug regimen received, a baseline CSF WBC count of $\geqslant$100 cells/μL, presence of trypanosomes in CSF, and a LATEX/IgM titer of $\geqslant$1:16 were found to be risk factors for treatment failure, a finding that corresponds to observations noted elsewhere [4, 16, 18, 23]. Although treatment failure was more common among HIV-positive patients, the difference was not significant.

To prevent serious sequelae resulting from late detection of trypanosomes during follow-up, it has been suggested to administer rescue treatment to patients for whom treatment failure (ie, those who are trypanosome negative) is suspected on the basis of indirect markers. The WBC count, LATEX/IgM titer, and trypanosome-specific antibody titer in CSF have been identified as markers for treatment failure [4, 24, 25], and we investigated their potential for earlier assessment of treatment outcome. At 3 months of follow-up, diagnosis of treatment failure should exclusively be based on trypanosome detection

Table 4. Sensitivity and Specificity of the Cerebrospinal Fluid White Blood Cell (WBC) Count for Detection of Treatment Failure in Patients with Second-Stage Gambiense Sleeping Sickness, by Different Follow-Up Time Points and Different Cutoff Values

Time after treatment	Patients with relapse, no.	Patients with a cure, no.	AUC (95% CI)	Cutoff WBC count, cells/μL	Sensitivity	Specificity	Youden Index
3 months	124	137	0.86 (0.82–0.91)	>30	0.73	0.88	0.61
				>5	0.96	0.32	0.28
				>20	0.80	0.77	0.57
				⩾50	0.57	0.95	0.52
6 months	77	136	0.93 (0.89–0.97)	>25	0.83	0.96	0.79
				>5	0.95	0.60	0.55
				>20	0.83	0.90	0.73
				⩾50	0.70	0.99	0.69
12 months	23	127	0.92 (0.82–1.00)	>23	0.87	1.00	0.87
				>5	0.91	0.87	0.79
				>20	0.87	0.99	0.86
				⩾50	0.52	1.00	0.52
18 months	9	108	0.92 (0.77–1.00)	>29	0.89	1.00	0.89
				>5	0.89	0.94	0.83
				>20	0.89	1.00	0.89
				⩾50	0.33	1.00	0.33
24 months	3	133	0.85 (0.55–1.00)	>36	0.67	1.00	0.67
				>5	0.67	0.99	0.66
				>20	0.67	1.00	0.67
				⩾50	0.00	1.00	0.00

NOTE. AUC, area under the curve; CI, confidence interval.

(ie, relapse), because we previously observed that relying on the CSF WBC count may result in many false-positive results [5], even if, in the present study, the WBC count had good discriminatory power at that time point. For clinical trials, the WHO recommends choosing between a follow-up assessment at 3 or 6 months, depending on the expected efficacy of the study drug [3]. At 6 months of follow-up, the WBC count is definitely more reliable than that noted at 3 months, with an AUC of 0.93. At 12 months of follow-up, both the WBC count and the LATEX/IgM titer could be used, but the AUC value of the latter is inferior. For CSF trypanosome-specific antibodies, the maximal Youden index was observed at 12 months but was only 0.43. Consequently, the CSF WBC count was the marker that distinguished cures from treatment failures soonest and most accurately.

A marker for cure could allow shortening of the duration of follow-up of patients with a low risk of treatment failure [4, 24], thus leading to a considerable workload reduction. We investigated, for the first time, the usefulness to follow-up of a combination of a criterion for treatment failure and a criterion for cure based on 2 markers, presence of trypanosomes, and the CSF WBC count, distinguishing 3 patient groups. Group 1 patients with a WBC count of ⩽5 cells/μL from 6 months onward and no trypanosomes detected are at low risk for treatment failure and are not to return for additional follow-up [4]. Group 2 patients with a WBC count of ⩾50 cells/μL and/or detection of trypanosomes at any follow-up assessment are considered to have treatment failure [5]. Group 3 patients with a WBC count of 6–49 cells/μL and with no trypanosomes detected are considered to have "uncertain evolution status" and should be told to attend the next scheduled follow-up assessment. For ToC assessment, a cutoff value of >20 cells/μL is used, as in other studies [3].

For patients with first-stage disease, the algorithm analysis could not go beyond 6 months, when follow-up would have been terminated in 34 of 36 patients and continued in 2 patients, with a sensitivity of 33.3% and a specificity of 100%. Because these 2 patients were, in reality, considered to have probable relapses, rescue treatment was administered, and no follow-up data were available after 6 months. Therefore, algorithm analysis was restricted to patients with second-stage disease. Application of algorithm B or C would result in a considerable reduction in the number of patients needing further follow-up (beyond 18 and 12 months, respectively) and shorter duration of follow-up. Because their sensitivity was identical, and because the difference in specificity was not statistically significant ($P = 1$), algorithm C, with a ToC assessment at 12 months, seems to be most appropriate for HAT

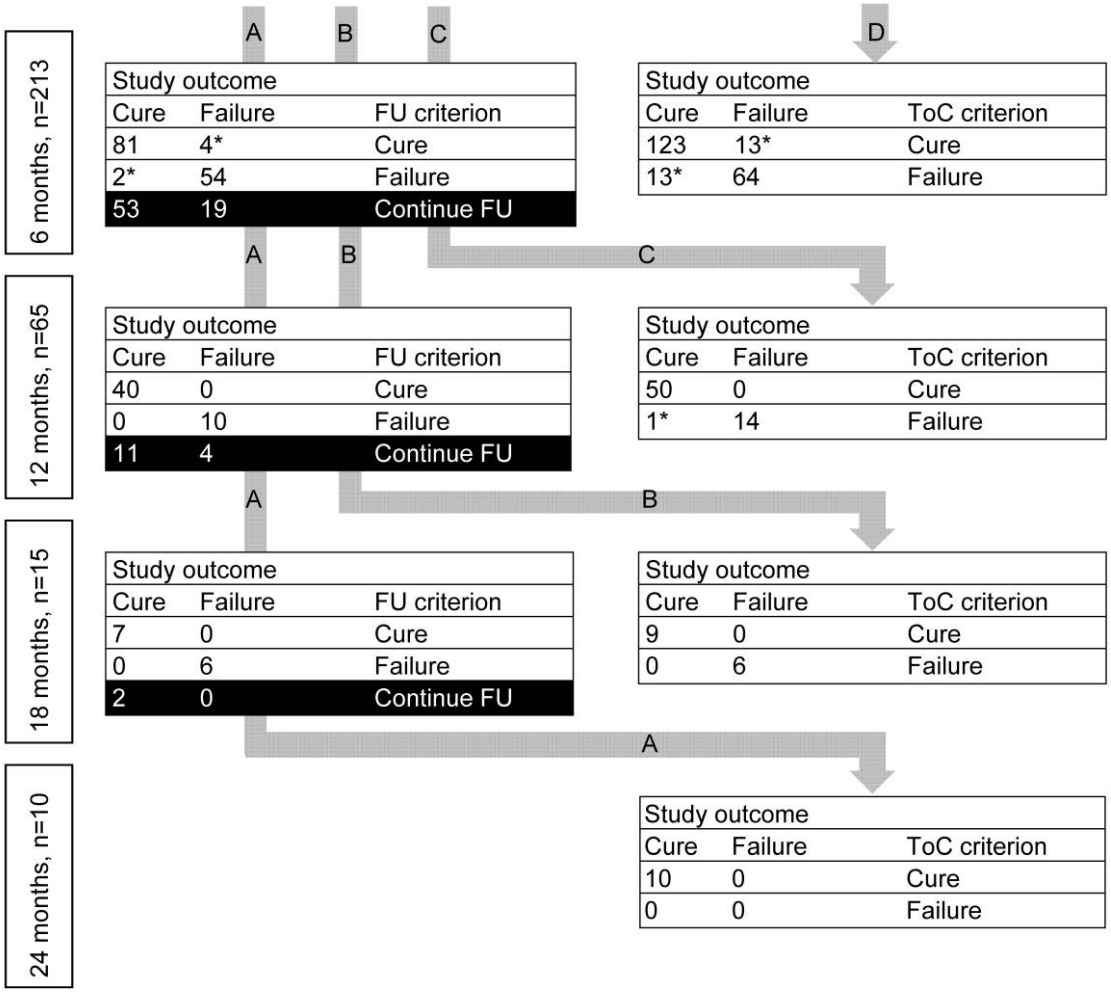

Figure 3. Effect of 4 algorithms *(A–D)* on the duration of follow-up (FU) of enrolled patients with second-stage human African trypanosomiasis (HAT), on the basis of a combination of 2 different criteria for treatment failure and cure. The FU criteria were as follows: for cure, ⩽5 white blood cells (WBCs)/μL cerebrospinal fluid (CSF) and no trypanosomes detected; for failure, ⩾50 WBCs/μL or presence of trypanosomes; for continued FU, 6–49 WBCs/μL and no trypanosomes detected. The criteria for test-of-cure (ToC) assessment were as follows: for cure, ⩽20 WBCs/μL and no trypanosomes detected; for failure, >20 WBCs/μL or presence of trypanosomes. *Wrongly classified outcome (not corresponding with real treatment outcome).

control and clinical trials. This algorithm showed a high specificity on both data sets on which it was tested. With sensitivities of 79% and 94%, a risk of missing treatment failures remains; therefore, patients classified as cured should be instructed to return to follow-up if they feel sick.

Our study had the following limitations. Although several investigated markers had cutoffs based on studies of other foci, and although many of our observations were similar to those of other investigators, our conclusions are based on a cohort in Mbuji Mayi, where the treatment failure rate noted after M monotherapy was unusually high. When we validated our algorithms in a second cohort with lower treatment failure rates, a lower sensitivity was obtained, although the difference was not significant. We were constrained to restrict our algorithm analysis to patients with second-stage disease, because the number of patients with first-stage disease was low. Our analysis comprised only field-applicable tests, whereas more-sophisticated techniques (like polymerase chain reaction, inflammation-related proteins, and/or brain damage markers [26]) might allow reducing the follow-up duration even further. Patients experiencing relapse after previously receiving HAT treatment were included in the cohort. The majority of our patients were treated with M, which increasingly is being replaced by E or N-E combination therapy. Because the timing of treatment failure might be drug regimen dependent, it may be useful to assess the efficacy of the proposed follow-up strategy retrospectively and/or prospectively in other patient cohorts before implementing it on a large scale.

In conclusion, by using simple trypanosome detection and the CSF WBC count as follow-up criteria at 6 months and by

introducing "no trypanosomes and ⩽20 WBCs/μL CSF" as criteria for cure at 12 months, posttreatment follow-up of patients with second-stage *gambiense* HAT may be considerably reduced, from the current 2-year duration to a maximum duration of 12 months. With fewer lumbar punctures (a 74% reduction), overall patient comfort improves considerably.

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