# Risk factors for treatment failure after melarsoprol for *Trypanosoma brucei gambiense* trypanosomiasis in Uganda

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**Abstract**

We evaluated the treatment failure rate among late-stage human African trypanosomiasis (HAT) patients treated with melarsoprol in Arua, northern Uganda, between September 1995 and August 1996, and identified the risk factors for treatment failure. We conducted a retrospective cohort study in October 1998, and performed a survival analysis. A treatment failure was defined as a late-stage HAT patient fully treated with melarsoprol and classified as an HAT case at any follow-up visit within 2 years after treatment. Among 428 patients treated in the study period, 130 (30·4%) were identified as treatment failure within 2 years after discharge. The multivariate analysis showed that patients who experienced treatment failure after melarsoprol were more likely to have been admitted as a relapsing case (relative hazard, RH = 11·15 [6·34–19·61]), and to have been diagnosed with trypanosomes in the lymph nodes (RH = 3·19 [2·10–4·83]) or in the cerebrospinal fluid (CSF) (RH = 1·66 [1·09–2·53]). The risk of treatment failure also increased with the number of cells in the CSF. The treatment failure rate after melarsoprol observed in Arua is greatly above the expected figures of 3–9%. More research is needed to confirm whether it is related to the variation of melarsoprol pharmacokinetics between individuals, or if it is associated with a reduced susceptibility of the trypanosomes to melarsoprol. The study emphasizes the need for second-line drugs to treat patients that have already received one or several full course(s) of melarsoprol.

**Keywords:** trypanosomiasis, *Trypanosoma brucei gambiense*, chemotherapy, melarsoprol, treatment failure, Uganda

## Introduction

In September 1995, the Ugandan National Sleeping Sickness Control Programme (NSSCP) with the assistance of Médecins sans Frontières (MSF) started a new control programme for human African trypanosomiasis (HAT) in the district of Arua, located in the focus of northwestern Uganda. This focus re-emerged after the major political disruptions experienced by the country during the 1970s and 1980s (MBULAMBERI, 1989a, 1989b).

The usual proportion of treatment failures after melarsoprol for cases of late-stage *Trypanosoma brucei gambiense* HAT is thought to be between 3% and 9% (PÉPIN *et al.*, 1994). However, a high proportion of HAT relapsing cases, previously treated with melarsoprol, was admitted in the NSSCP/MSF treatment centre in Arua. We therefore conducted a study to evaluate the treatment failure rate after melarsoprol among late-stage HAT patients treated in Arua, and to identify the risk factors for treatment failure.

## Methods

Before treatment, patients were classified in the late-stage of HAT (corresponding to nervous system involvement) if the trypanosome was isolated in the cerebrospinal fluid (CSF), or only in blood or lymph-node fluid but with more than 5 cells/mm$^3$ in the CSF. Patients with more than 20 cells/mm$^3$ in the CSF were also considered late-stage patients, even if no trypanosome was isolated at all. At the time of their admission, patients were categorized as 'new cases' if they were receiving their first treatment, or as 'relapsing cases' if they had already been treated for HAT. Late-stage cases of HAT were treated with 3 courses of 3 injections of melarsoprol, each along with oral prednisolone. Prednisolone was started on the first day of hospital admission. The following schedule was used for melarsoprol: 1·8, 2·16 and 2·52 mg/kg on days 2, 3 and 4—6 days of rest from day 5 to day 10—2·52, 2·88 and 3·25 mg/kg on days 11, 12 and 13—6 days of rest from day 14 to day 19—3·6 mg/kg on days 20, 21 and 22. After treatment completion, 3 follow-up visits at 6, 12 and 24 months after discharge were scheduled. A glandular puncture (GP) (if lymph nodes were present) and a lumbar puncture were performed at each of those visits. Patients were then classified as treatment failure if the trypanosome was isolated in the CSF or in the lymph-node fluid, or if the number of cells in the CSF increased as compared to the CSF cell count before treatment.

Late-stage HAT patients treated with melarsoprol during the first year of the programme (i.e., September 1995–August 1996), who completed their treatment and were discharged alive from the hospital, were included in a retrospective cohort study conducted in October 1998.

We first determined the proportion of patients identified as treatment failure among those included in the cohort. Survival analysis methods were then used to identify the risk factors for treatment failure within 24 months after hospital admission. The date of discharge was chosen as time 0, the event was the treatment failure. The date of failure was chosen as the mid-interval between the date of the treatment failure diagnosis, and the date of the last negative visit (or the date of discharge when the failure was diagnosed at the first follow-up visit). Patients who died less than 6 months after completing their treatment (e.g., without coming to any follow-up visit) were considered to be treatment failures at 3 months. Among patients not identified as treatment failure, those followed-up for 24 months or more were censored at 24 months, and those lost to follow-up before 24 months were censored at the time of their last negative visit. Patients, not reported deceased, who did not complete any follow-up visit were excluded from the analysis.

The Kaplan–Meier method was used for the univariate analysis, and the logrank test to compare the exposure groups. In order to adjust for the effects of potential confounders, we performed a multivariate survival analysis using the Cox proportional hazard model, and the relative hazard (RH) as an estimation of the relative risk. The following variables of exposure, recorded during the hospital stay, were included in the Cox models: age, sex, presence of trypanosome(s) in the lymph nodes, presence of trypanosome(s) in the CSF, number of cells in the CSF, admission as a new or as a relapsing case, and any side-effects during treatment. Patients with no palpable cervical nodes were considered negative for GP. Among side-effects, the melarsoprol reaction was defined as the occurrence of tachycardia (pulse > 100, or > 140 in patients aged under 12 years), associated with fever (> 38°C) and perilimbal conjunctival hyperaemia.

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The reactive encephalopathy to melarsoprol was defined by the occurrence of a sudden episode of coma or status epilepticus. The survival functions on a $\log_e$ ($-\log_e$) scale were plotted over time for each covariate included in the model to check whether the assumption of proportional hazards was met. Data were analysed with EpiInfo (CDC, Atlanta, USA) and SPSS (SPSS Inc., Chicago, USA) software packages.

## Results

Among 473 late-stage HAT patients treated with melarsoprol between September 1995 and August 1996, 19 (4·0%) died and 1 ran away during hospital stay. Twenty-five patients (5·5%), not reported as deceased, failed to come to any follow-up visit and were excluded. Of the remaining 428 patients, 130 (30·4%) were classified as treatment failure, including 15 who died before completing their first follow-up visit. Among the patients not classified as treatment failure, 174 (58·4%) were lost to follow-up before 24 months, their length of follow-up ranging from 178 to 729 days (median 456 days).

Patients admitted as relapsing cases had a higher treatment failure rate than those admitted as new cases (91·3% vs 26·9%, $P < 0.0001$) (Table 1). Most of the treatment failure observed among patients admitted as relapsing cases ($n = 23$) occurred during the first year of follow-up (Figure), only 1 had a negative follow-up visit at 24 months, and 1 was lost to follow-up after 12 months. The treatment failure rate was also higher among patients diagnosed with trypanosomes in the lymph nodes (39·1% vs 24·0% for patients with negative GP, $P = 0.002$) or in the CSF (36·2% vs 20·4% for patients with no trypanosomes in CSF, $P = 0.0002$), and it tended to increase with the number of cells in the CSF (Table 1 and Figure).

The multivariate analysis confirmed that patients who experienced treatment failure after melarsoprol were more likely to have been admitted as relapsing cases (RH = 11·15 [6·34–19·61]), and to have been diagnosed with trypanosomes in the lymph nodes (RH = 3·19 [2·10–4·83]) or in the CSF (RH = 1·66 [1·09–2·53]) (Table 2). The risk of treatment failure also increased with the number of cells in the CSF. By contrast, age and gender had no significant influence. The occurrence of melarsoprol reaction during hospital stay had a protective effect, but the RH was not statistically significant (0·59 [0·30–1·14]).

## Discussion

The observed proportion of treatment failures among patients treated with melarsoprol for *T. b. gambiense* trypanosomiasis in Arua (30·4%) is greatly above the expected figures of 3–9% failure rate (PÉPIN *et al.*, 1994). While a high treatment failure rate was expected among patients admitted as relapsing cases (PÉPIN & MILORD, 1994), the proportion of failures observed among patients treated for the first time in our series (26·9%) remains very unusual. In fact, the treatment failure rate observed in Arua is much higher than that observed in Adjumani (0·4–2·5%, depending on the year), a neighbouring district, between 1991 and 1996 (BOUCHET *et al.*, 1998). Yet, the 2 programmes are run by the same organizations (NSSCP/MSF), the climatic conditions are similar, and the same laboratory techniques and treatment protocols and the same lots of melarsoprol were used.

Some potential biases could explain our result. A high proportion of patients did not complete their 24-month follow-up. We might then have missed some failure cases, and therefore, underestimated the true treatment failure rate. On the other hand, the methods used to classify patients as treatment failure (trypanosomes or increased number of cells in the CSF) could result in an overestimation of the true treatment failure rate. However, out of 115 patients identified with treatment failure,

**Table 1. Treatment failure rates observed after 2 years among late-stage human African trypanosomiasis patients treated with melarsoprol by group of exposure, Arua, Uganda, September 1995–October 1998**

| Variables at time of admission | *n* | Treatment failure rate after 2 years (%) | *P*[a] |
|---|---|---|---|
| Age (years) | | | |
| 0–14 | 87 | 36·8 | 0·35 |
| 15–29 | 188 | 30·9 | |
| ⩾ 30 | 153 | 26·1 | |
| Sex | | | |
| Female | 233 | 28·8 | 0·67 |
| Male | 194 | 32·0 | |
| Mode of admission | | | |
| New cases | 405 | 26·9 | < 0·0001 |
| Relapsing cases | 23 | 91·3 | |
| Glandular puncture | | | |
| Negative | 250 | 24·0 | 0·002 |
| Positive | 174 | 39·1 | |
| Trypanosomes in CSF | | | |
| Negative | 157 | 20·4 | 0·0002 |
| Positive | 271 | 36·2 | |
| Number of cells in CSF (per $mm^3$) | | | |
| ⩽ 20 | 54 | 16·7 | 0·001 |
| 21–100 | 140 | 24·3 | |
| 101–200 | 108 | 38·0 | |
| ⩾ 201 | 122 | 37·7 | |
| Side-effects | | | |
| None | 304 | 30·6 | 0·69 |
| Moderate[b] | 72 | 30·6 | |
| Melarsoprol reaction | 40 | 25·0 | |
| Encephalopathy | 12 | 41·7 | |

CSF, cerebrospinal fluid.
[a]From a logrank test.
[b]Cellulitis, enteritis, arrhythmia, peripheral neuropathy.

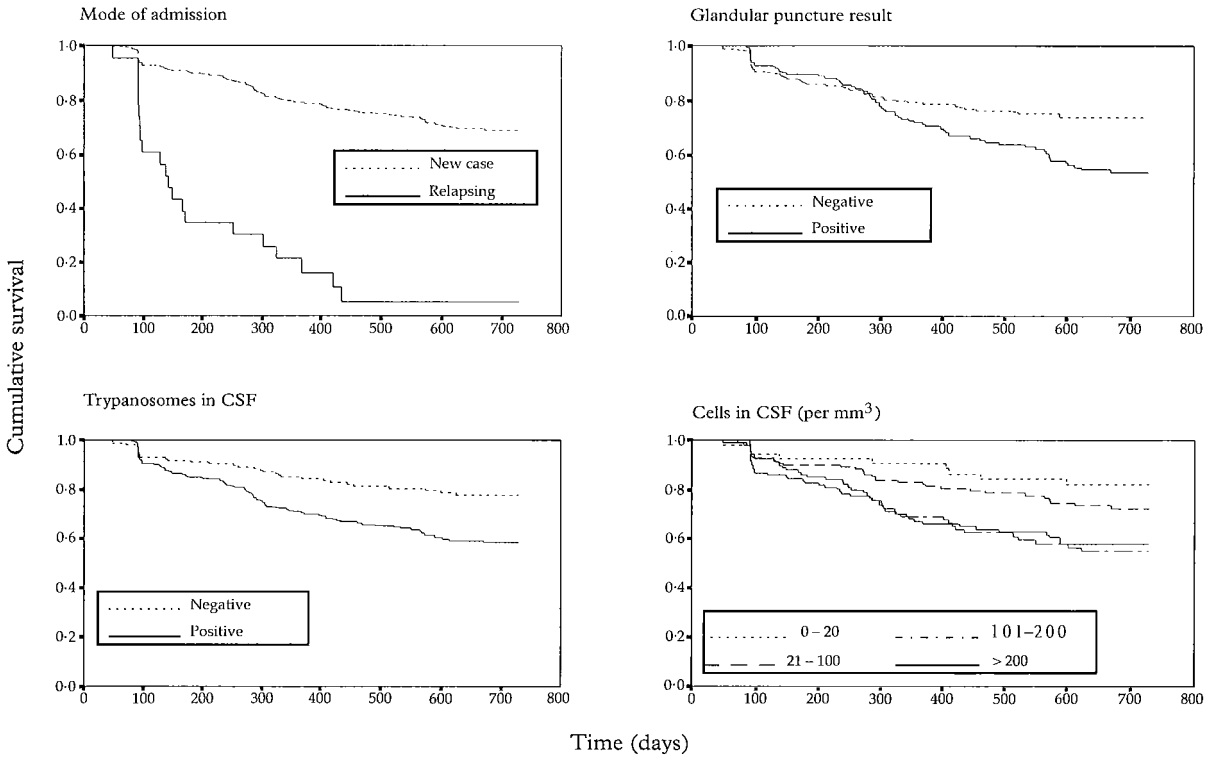


Figure. Two years cumulative survival curves among late-stage human African trypanosomiasis patients treated with melarsoprol by group of exposure, for selected variables, Arua, Uganda, September 1995–October 1998. CSF, cerebrospinal fluid.

**Table 2. Relative hazard (RH) estimates for treatment failure after 2 years among late-stage human African trypanosomiasis patients treated with melarsoprol by group of exposure, Arua, Uganda, September 1995–October 1998**

| Variables at time of admission | *n* | RH[a] [95% CI] (univariate) | RH[a] [95% CI] (multivariate) |
|---|---|---|---|
| Age (years) | | | |
| 0–14 | 87 | 1·40 [0·88–2·23] | 0·97 [0·58–1·60] |
| 15–29 | 188 | 1·21 [0·81–1·81] | 1·15 [0·76–1·76] |
| ⩾ 30 | 153 | 1·00 | 1·00 |
| Sex | | | |
| Female | 233 | 1·00 | 1·00 |
| Male | 194 | 1·10 [0·90–1·34] | 1·05 [0·83–1·31] |
| Mode of admission | | | |
| New cases | 405 | 1·00 | 1·00 |
| Relapsing cases | 23 | 7·42 [4·59–12·0] | 11·15 [6·34–19·61] |
| Glandular puncture | | | |
| Negative | 250 | 1·00 | 1·00 |
| Positive | 174 | 1·74 [1·23–2·46] | 3·19 [2·10–4·83] |
| Trypanosomes in CSF | | | |
| Negative | 157 | 1·00 | 1·00 |
| Positive | 271 | 2·07 [1·39–3·09] | 1·66 [1·09–2·53] |
| Number of cells in CSF (per mm$^3$) | | | |
| ⩽ 20 | 54 | 1·00 | 1·00 |
| 21–100 | 140 | 1·59 [0·76–3·32] | 1·65 [0·76–3·60] |
| 101–200 | 108 | 2·92 [1·42–6·02] | 3·25 [1·48–7·14] |
| ⩾ 201 | 122 | 2·84 [1·39–5·80] | 3·41 [1·56–7·46] |
| Side-effects | | | |
| None | 304 | 1·00 | 1·00 |
| Moderate[b] | 72 | 0·95 [0·60–1·52] | 0·91 [0·56–1·48] |
| Melarsoprol reaction | 40 | 0·75 [0·39–1·45] | 0·59 [0·30–1·14] |
| Encephalopathy | 12 | 1·43 [0·58–3·53] | 1·32 [0·53–3·29] |

CSF, cerebrospinal fluid.
[a]Relative hazard, estimated from the Cox proportional hazard model, with confidence intervals.
[b]Cellulitis, enteritis, arrhythmia, peripheral neuropathy.

96 (89·5%) were diagnosed with trypanosomes in CSF. We can therefore assume that some false-positive diagnoses might be present, but that they would hardly explain our results. A major re-infection phenomenon is also unlikely given the proportion of failure cases found with trypanosomes in CSF and the low level of *T. b. gambiense* transmission in Arua. Finally, a quality control exercise performed in the laboratory and in the patient ward confirmed that the laboratory and nursing procedures were being respected.

Some of the risk factors for treatment failure identified in our series, such as the presence of trypanosomes or an increased number of cells in the CSF, have been already reported from other settings (PÉPIN *et al.*, 1994). However, a positive GP at the time of admission was not expected to be a risk factor for treatment failure. This result could be related to the unusually high proportion of patients with positive GP in our group of late-stage cases (40·7%). Regarding the occurrence of side-effects during hospital stay, the interpretation of their role on the risk of treatment failure has to be made with caution. In fact, the milder cases of melarsoprol reaction or encephalopathy were selected, because we excluded the 19 patients who died during hospital stay, and 12 of them (63·2%) died after developing an encephalopathy.

A phenomenon of resistance to melarsoprol among the trypanosomes circulating in Arua is one of the hypotheses that could explain the high treatment failure rate observed. However, to our knowledge, strains of trypanosomes resistant to melarsoprol have never been isolated. The variation of melarsoprol pharmacokinetics between individuals could also explain the occurrence of the treatment failures, particularly if associated with a reduced susceptibility of the trypanosomes. Considering the low levels of melarsoprol in the CSF of patients treated with intermittent courses (as used in Uganda) (BURRI *et al.*, 1993), the characteristics of the individual (particularly the impairment of the blood–brain barrier) could prevent the drug from reaching an adequate concentration in the CSF.

The occurrence of this high treatment failure rate in a focus of HAT emphasizes the need for second-line trypanocidal drugs. Currently, only α-difluoromethylornithine (eflornithine) has proven efficacy to replace melarsoprol in the treatment of late-stage HAT patients (MILORD *et al.*, 1992; DOUA & BOA YAPO, 1993). However, this drug will no longer be produced unless 5000 ampoules are purchased and paid for in advance at the prohibitive cost of US$60 an ampoule, which represents $840 for a full treatment of 14 days. Nifurtimox has been tested in several places with contradictory results (PÉPIN *et al.*, 1992; VAN NIEUWENHOVE, 1992; DOUA & BOA YAPO, 1994). Its use as a second-line drug cannot be recommended unless extensive investigations, including clinical trials, are performed.

At the same time, more research is needed to fully investigate the origin of the treatment failures. The level of resistance *in vitro* of the strains of trypanosomes and the pharmacokinetic characteristics of melarsoprol are the main factors that require further elucidation. This type of research is difficult to conduct in the field. The isolation of strains of trypanosomes, in particular, requires rat inoculation or cryopreservation on the spot. New techniques, accessible for routine use, should be developed.

**Acknowledgements**

This study was conducted with the financial support of the European Development Fund and Médecins sans Frontières. We thank the personnel of the trypanosomiasis treatment centre in Arua for their participation in the study.

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*Received 28 July 1998; revised 5 March 1999; accepted for publication 23 March 1999*

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