

Treatment and follow-up using microscopy and polymerase chain reaction in East African sleeping sickness: a case report

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Date accepted for publication 17 January 2011

Abstract

We report a case of East African trypanosomiasis in a 26-year-old traveler returning from Tanzania, including a series of pictures of the progression of the inoculation chancre. *Trypanosoma brucei rhodesiense*-specific DNA was detected in the blood until 8 days after treatment. We hypothesize that the previously observed suramin toxicity may be an immunological response to parasite debris, produced as they are killed by the treatment.

Keywords

Trypanosoma brucei rhodesiense; sleeping sickness; suramin.

Introduction

East African sleeping sickness is caused by *Trypanosoma brucei rhodesiense* and is transmitted by the bite of the tsetse fly (genus *Glossina morsitans*)^[1]. Human African trypanosomiasis (HAT) can be categorized into West and East African trypanosomiasis and is endemic in sub-Saharan Africa with a population at risk of about 60 million people^[2,3]. East African sleeping sickness is rarely seen in travelers returning to a non-endemic area; only 21 cases have been reported in travelers returning from the United States in the 20th century^[4,5]. In 2001, a cluster of 9 patients was described with *T.b. rhodesiense* after traveling to Tanzanian game parks^[6] and between 2005 and 2009 only 5 cases of East African sleeping sickness were reported to Eurosurveillance^[7]. A few case reports have been published more recently^[8–9].

A timely diagnosis and correct staging of the disease are important because HAT is a fatal disease for which treatment can be lifesaving. A Giemsa-stained thick blood smear or a quantitative buffy coat test (QBC) can be used for the detection of trypanosomes in the blood.

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Recently, a real-time polymerase chain reaction (PCR) for the detection of *Trypanosoma brucei* DNA in human blood samples has been described, with a lower detection limit of 100 trypanosomes per milliliter of blood^[10]. After inoculation, often resulting in a chancre, the trypanosomes multiply in blood and lymph, causing the first stage of HAT. In the second stage, the central nervous system is invaded by trypanosomes and without treatment this leads to apathy, coma and death. In *T.b. rhodesiense* the incubation period is 1-4 weeks^[4]. Invasion of parasites in the cerebrospinal fluid (CSF) usually occurs within 3 months of inoculation.

For the treatment of East African sleeping sickness, only suramin and melarsoprol are used in the first and second stage of the disease, respectively. Suramin is a polysulfonated naphthylamine polyanionic compound first used in 1922^[2,11]; its mechanism of action is still not fully understood. Because it is highly ionic in nature, it cannot penetrate into the CSF. A test dose is administered to reveal idiosyncratic reactions. Immediate and life-threatening side effects of suramin, such as collapse and shock^[2,12] have been described, as well as delayed reactions including renal failure, exfoliative dermatitis, agranulocytosis, hemolytic anemia, jaundice and severe diarrhea^[2]. Melarsoprol is a derivative of arsenic and has been used since 1949^[11,13]. It penetrates into the CSF, but the mechanism of action of this drug is also not yet fully understood. The worst adverse event is reactive encephalopathy, which leads to hyperthermic coma and death within 48 h in 10–50% of affected patients. This reaction is mostly believed to be due to massive destruction of trypanosomes in the central nervous system and not to a direct toxic effect of melarsoprol^[11,12].

In the present report the use of PCR is illustrated in a recently encountered case of East African sleeping sickness. This case suggests that suramin toxicity may be an effect of trypanosomal decay rather than the result of direct toxicity.

Case report

A 26-year-old woman with an unremarkable medical history presented to the emergency room with fever, chills and cellulitis of the left arm. A day earlier, she had returned from Tanzania, where she had spent her honeymoon. She traveled for 3 weeks and had visited the Serengeti National Park. The couple always slept in hotels. She recalled being bitten by grey flies during this visit. These bites were not particularly painful. Four days before presentation our patient had noticed a painful reddish swelling on her left arm, which evolved into a blister. She was also suffering from a retro-orbital headache. Her medication consisted of atovaquon/proguanil as malaria prophylaxis and an oral contraceptive.

On physical examination, she did not appear ill and had a fever of 39.4°C, blood pressure of 140/70 mmHg and a pulse of 108 bpm. On the left forearm a well-demarcated, indurated, red nodule measuring 10×5 cm was seen with a central bulla measuring 2.5 cm in diameter (Fig. 1). Further physical examination was unremarkable. There were no neurological abnormalities or lymphadenopathy.

Laboratory studies showed a leukocytosis of $5.4 \times 10^9/l$ with 13% band neutrophils and a serum C-reactive protein level of 62 mg/l without other abnormalities (Table 1). Cellulitis due to *Staphylococcus aureus* was suspected and intravenous flucloxacillin was started. The following day there had been an expansion of the erythema on her arm with adjacent lymphangitis, but without axillary lymphadenopathy. A headline diagnosis of an inoculation chancre of East African sleeping sickness was considered, which was confirmed by a thick smear and QBC, revealing trypanosomes (Fig. 2). Cultures of blister fluid and blood remained sterile. A lumbar puncture was

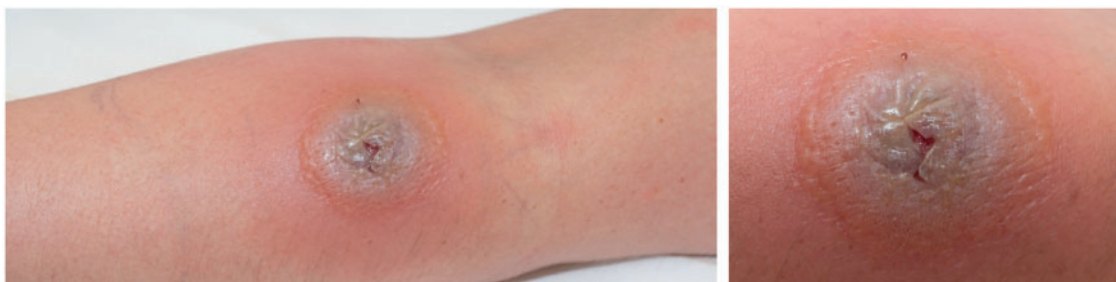
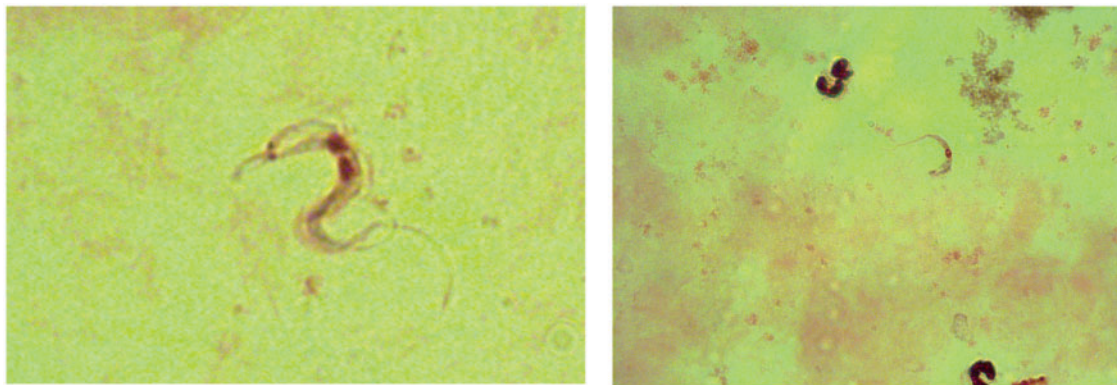


Fig. 1. Inoculation chancre day 1.

Table 1. Laboratory findings

| | Initial findings | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Units |
|-------------|------------------|-------|-------|-------|-------|-------|-------|-------|-------------------|
| Hb | 8.7 | 8.1 | 7.2 | 7.1 | 6.7 | 7.3 | 7.8 | 7.5 | mmol/l |
| WBC | 5.4 | 5.0 | 2.3 | 2.9 | 3.8 | 4.4 | 12.9 | 6.4 | $\times 10^9/l$ |
| Platelets | 279 | — | 45 | 45 | 50 | — | 439 | — | $\times 10^9/l$ |
| Creatinine | 64 | 62 | 68 | 50 | 45 | 48 | 47 | 59 | $\mu\text{mol/l}$ |
| Bilirubin | | 22 | 48 | 49 | 43 | 25 | 17 | 8 | $\mu\text{mol/l}$ |
| ALP | 63 | 57 | 71 | 33 | 187 | 316 | 208 | 63 | U/l |
| GammaGT | 80 | 219 | 350 | 321 | 443 | 679 | 435 | 90 | U/l |
| ASAT | 31 | 97 | 232 | 235 | 370 | 335 | 60 | 22 | U/l |
| ALAT | 28 | 66 | 135 | 177 | 274 | 315 | 146 | 29 | U/l |
| LDH | 176 | 325 | 506 | 508 | 447 | 392 | 279 | 143 | U/l |
| Proteinuria | — | ++ | ++ | Trace | Trace | + | — | Trace | |

Hb, hemoglobin; WBC, white blood cell count; ALP, alkaline phosphatase; gammaGT, gamma-glutamyl transpeptidase; ASAT, aspartate aminotransferase level; ALAT, alanine aminotransferase level; LDH, lactate dehydrogenase; +, proteinuria $>300\text{ mg/l}$, ++, $>1000\text{ mg/l}$ and $<3000\text{ mg/l}$.

**Fig. 2.** Flagellated trypanosomes visible in thick smear.

performed to assess the stage of the disease. There was no invasion of the central nervous system. Intravenous suramin was started for early stage *T.b. rhodesiense* infection. A test dose of 100 mg was administered, followed by 400 mg when there was no adverse reaction. The following day, an additional 500 mg was administered. Even before this additional dose, our patient had an acute deterioration with malaise, headache, photophobia, nausea, vomiting and diarrhea, hypotension of 66/45 mmHg and an erythematous targetoid rash of the head, neck and trunk. Laboratory studies revealed a pancytopenia, proteinuria and increased liver enzymes, all of which improved in the following days (Table 1). The symptoms disappeared over the course of 2 days. After the first gram, our patient received 5 more doses of 1 g at weekly intervals^[12], without any clinical reaction. Because the reaction occurred almost 15 h after the second dose of suramin was given, an immunological reaction related to parasite death, comparable with a Jarisch-Herxheimer reaction provoked by treatment of spirochetes^[14], was suspected. Levels of tryptase and circulating immune complexes were determined in the earliest serum sample available, taken 5 days after the first dose of suramin. Both levels were increased (tryptase $51\text{ }\mu\text{g/l}$ [ref: 0–20]; circulating immune complexes: $9.3\text{ }\mu\text{g/ml}$ [ref: 0.0–4.1]). No more trypanosomes were detected in the thick smear after 2 days. The QBC showed trypanosomes until 2 days after the first dose of suramin. For 9 more days small fluorescent structures were seen in the QBC, but they were not recognizable as trypanosomes. Real-time PCR, as described by Becker et al.^[10], was retrospectively performed on CSF and blood samples. In the CSF no *T. brucei*-specific DNA was detected. PCR was positive in the blood until day 8 after the start of treatment. During this period the DNA load decreased. A slight increase of the DNA load was detected on days 2 and 8. From day 9 onwards

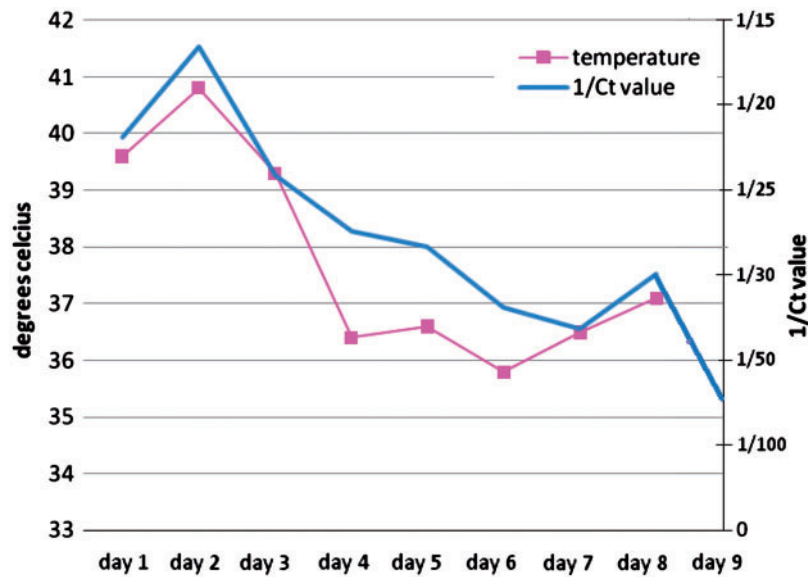


Fig. 3. PCRs for *T. brucei* DNA (represented by 1/cycle threshold (CT) value) and temperature in the course of the disease.



Fig. 4. Progression of chancre, day 5, day 8 and day 36, respectively.

the PCR remained negative (Fig. 3). The chancre gradually healed within 4 weeks, without leaving a scar (Fig. 4). After 1-year follow-up there were no signs of relapse and the patient was feeling well.

Discussion

Our patient had visited the Serengeti National Park in Tanzania, where she noted many tsetse flies. She is one of 5 patients reported to Eurosurveillance with East African sleeping sickness between 2005 and 2009^[7]. This illustrates that however rare, one should always consider sleeping sickness in travelers returning from endemic areas, in particular when they present with a prominent chancre like our patient. In 2001 a cluster of 9 European and South African patients was reported after visiting Tanzania^[6], whereupon efforts were intensified to reduce the local population of tsetse flies. Therefore, it is worthwhile reporting new cases of East African sleeping sickness to identify new clusters and possibly intensify actions against tsetse flies.

After starting treatment, rapid clearance of trypanosomes from the blood, lymph and peripheral tissue is thought to occur. In our case, *T. brucei* DNA was detected using real-time PCR until day 8 after the start of treatment, whereas in the thick smear and the QBC, no more trypanosomes could be found after 2 days. In the QBC, small stained structures were seen for a longer period. It is unclear whether this suggests a sort of debris after destruction of trypanosomes or a prolonged presence of parasites in the body cavities. It could be speculated that the increase in DNA load on days 2 and 8 suggest the latter.

Approximately 15 hours after the test dose of suramin, our patient developed hypotension, an erythematous rash and a high fever. This may have been a toxic reaction to suramin^[2,12], but

these reactions normally occur immediately after administration. Moreover, none of the following injections caused any reaction. Another explanation may be that lysis of trypanosomes triggered an immunological response, comparable with the Jarisch–Herxheimer reaction^[14]. The finding of a high serum tryptase level, indicative of mast cell activation, and circulating immune complexes might be interpreted as circumstantial evidence for such an immunological reaction. We found no other case reports describing such reactions, possibly because the symptoms are easily ascribed to drug toxicity or progression of the disease.

Teaching Points

- Continuing awareness of this disease, which is rare in travelers but uniformly fatal if untreated, is necessary.
- PCR is a sensitive method for diagnosis and follow-up after treatment of trypanosomiasis.
- Drug toxicity may not be a dominant factor in reactions observed after the administration of suramin.

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