Chagasic Meningoencephalitis in a Patient with Acquired Immunodeficiency Syndrome: Diagnosis, Follow-Up, and Genetic Characterization of *Trypanosoma cruzi*

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Early diagnosis of the clinical reactivation of Chagas’ disease in human immunodeficiency virus– and *Trypanosoma cruzi*–coinfected persons is fundamental for a good prognosis. Polymerase chain reaction rapidly and efficiently demonstrated the presence and elimination of *T. cruzi* in the cerebrospinal fluid of a patient with chagasic meningoencephalitis. Characterization of *T. cruzi*, directly and indirectly in blood and cerebrospinal fluid samples, demonstrated homogeneity of kinetoplast DNA and the presence of lineage 1 (*T. cruzi* II) in both parasite populations.

Reactivation of chagasic infection does not occur spontaneously and has been associated with immunosuppression in patients who have undergone transplantation [1, 2], who have hematologic cancer [3, 4], and who have AIDS [5–9].

In patients coinfected with HIV, the reactivation of Chagas’ disease can occur, and it has been described in ~60 well-documented cases [10]. The factors involved in this association are not clear; they may be related to selective immune depletion or to characteristics of the parasite. These situations have been demonstrated by the presence of the parasite on buffy coat or microhematocrit [11, 12] and by the invasion of the CNS or the heart [7, 8, 11] in the majority of cases. Other less common manifestations are reported in the gastric and esophageal apparatus [8], skin lesions [13], peritoneum [14], and cervix uteri [15].

*Trypanosoma cruzi* invasion of the CNS during the chronic phase of chagasic infection may be associated with the presence of specific subpopulations. Experimental studies correlating phylogenetic divergences, histopathologic alterations, and tissue tropism have associated the genotype 20 of *T. cruzi* with CNS invasion [16]. Thus, it is important to evaluate whether this parasite presents defined genetic profiles that may be associated with neurotropism.

Current tests for diagnosis of CNS invasion in Chagas’ disease have a low sensitivity and, in some situations, they do not allow the establishment of etiology. Rapid and specific techniques, such as PCR, which detects minimal quantities of the parasitic DNA [17, 18], may be useful for early diagnosis of the disease and for monitoring of treatment.

This investigation demonstrates the applicability of molecular techniques to detect *T. cruzi* in blood and CSF specimens and for genetic characterization of the parasitic population associated with CNS invasion in humans.

**Case report and methods.** A 63-year-old man from Minas Gerais, Brazil, was hospitalized because of a history of fever that lasted several days, general malaise, and serological test results that were positive for HIV. Three days after he was hospitalized, the patient presented with monoparesis, which rapidly developed into hemiparesis. CT of the brain revealed a cerebral lesion on the right side. The diagnostic hypothesis was cerebral toxoplasmosis, and a therapeutic trial of sulfadiazine and pyrimethamine was initiated. Despite treatment, the clinical situation evolved to mental confusion, psychomotor agitation, nuchal rigidity, and paralytic mydriasis. At that time, the patient’s T lymphocyte count was 67 CD4+ cells/mm3 and 313 CD8+ cells/mm3, and electrocardiography revealed left atrial overload, left ventricular overload, and diffuse alteration of ventricular repolarization. Chagasic infection and reactivation was diagnosed 6 days after admission on the basis of serological test results and demonstration of parasites in samples of CSF and blood. Therapy with benznidazole (7 mg/kg/day for 60 days) was started, but the patient fell into a profound coma 3 days later. Treatment was monitored by parasitological evaluation of CSF samples and cranial tomography at 7 and...
22 days. Sixty-eight days after hospitalization, the patient was discharged from hospital, still in a comatose state.

At the time of hospitalization, the patient had signed a consent form authorizing all necessary procedures during his hospitalization and their use for teaching and research. This work was conducted in accordance with the ethical committee of Triângulo Mineiro Faculty of Medicine.

Samples of CSF and blood were obtained simultaneously. They were collected initially to confirm the diagnostic hypothesis (T0) and later to follow-up the patient at 7 (T1) and 22 days (T2) after the initiation of treatment. These samples were submitted to direct microscopic examination, microhematocrit examination, culture in liver infusion tryptose medium (LIT), and PCR for the detection of the parasite and/or its DNA. Hemoculture was done with 10 mL of blood in LIT, according to methodology described elsewhere [19]. Culture of CSF specimens was done by the addition of 1 mL of aspirated fluid directly onto 5 mL of LIT. Microscopic examination of the blood culture was done on days 30, 60, and 90.

Total blood (10 mL) and CSF (2 mL) samples were mixed with an equal volume of 6 M guanidine hydrochloride and 0.2 M of EDTA solution [17]. Samples that yielded negative results by direct or microhematocrit examination were boiled before extraction of the DNA for 15 min [20]; for all other samples, the DNA was extracted without previous boiling, and DNA extraction was done according to a method described elsewhere [18]. The blood or CSF culture samples were diluted 1:1 in guanidine-EDTA, and DNA extraction was done without boiling [18].

For all samples, specific PCR amplification of the 330-bp fragment corresponding to the 4 variable regions of *T. cruzi* kinetoplast (k) DNA was done with use of primers 121 (5′-AAATAATGTACGGG(T/G)GAGATGCATGA-3′) and 122 (5′-GGTTCCGATTGGGTGGTATATA-3′; both from Operon Technology) [18]. PCR products were visualized by 6% polyacrylamide gel (PAGE) and silver stained. For the CSF samples, PCR was done on successively diluted DNA samples until no amplified products could be detected in the polyacrylamide gels, followed by hybridization. For the samples in which no PCR amplification was detected, the presence of amplification inhibitors was verified by the addition to the samples of 0.1 ng of DNA obtained from parasites from culture.

Slot-blot hybridization was done with use of PCR-amplified DNA samples to confirm *T. cruzi* specificity for the amplicons observed in the polyacrylamide gels. The PCR products were denatured and applied to nylon membranes (Biodyne B; Life Technologies Gibco-BRL) by means of a slot-blot apparatus (Hofer Scientific Instruments). The membrane was hybridized for 30 min with the alkaline phosphatase–labeled oligonucleotide probe [21] S-67 (5′-TGGTTTTGGAAGGCGGTCTCAAATT-3′; synthesized by Lifecodes Corporation). Detection was done with chemiluminescence by use of a LumiPhos-Plus substrate (Life Technologies Gibco-BRL) and x-ray film exposure, as described elsewhere [18].

The kinetoplast genetic characterization of parasites was done directly from the samples and with parasites cultured from blood or CSF samples obtained before the initiation of treatment (T0) and 1 week later (T1). The first step consisted of PCR amplification of *T. cruzi* k-DNA. PCR-amplified products were submitted to electrophoresis on 1.5% agarose gels (1.0% agarose, 0.5% low-melting-point agarose) stained with ethidium bromide. Bands corresponding to the 330-bp fragment were excised from the gel. These were diluted to 1:10 in double-distilled water and submitted to a second step of low-stringency amplification with use of a single primer S35G (5′-AAATAATGTACGGGGAGGTGAATATA-3′; Operon Technology) [22]. The LSSP-PCR positive control was done with the CI strain of *T. cruzi* DNA.

PCR amplification of a divergent domain of the 24SαRNA gene was done with primers D71 (5′-AAGGTGAGTCAGACGTGGTGAATATA-3′) and D72 (5′-TTTTCAAGATGGCAGAACGT-3′; Operon Technology) on parasites cultured from blood and CSF samples obtained at T0, following protocols described elsewhere [23]. PCR products were visualized by 6.0% PAGE and silver stained. The amplification of a fragment of 125 bp corresponded to lineage 1, and a fragment of 110 bp corresponded to lineage 2, currently known as *T. cruzi* II and *T. cruzi* I, respectively [24].

**Results.** Examination of fresh samples obtained at T0 demonstrated 10³ parasites/mL in the blood and uncountable trypanomastigote forms in the CSF, which indicates strong invasion of the CNS. Treatment with benznidazole induced a dramatic reduction in the parasitic level in both the blood and the CSF; at T1, *T. cruzi* was detected by microhematocrit examination, and at T2, the parasite was not detected by use of any of the direct methods. Cultures of blood and CSF samples yielded parasites at T0 and T1 and were negative at T2.

The k-DNA of *T. cruzi* was strongly amplified in CSF at T0 and T1. Despite the reduction in parasite levels after treatment, the quantity of DNA in the CSF remained high at T1, with levels similar to those found at T0. PCR followed by hybridization demonstrated products corresponding to the amplification of *T. cruzi* k-DNA up to dilutions of 10⁻⁷ and 10⁻⁸ for T0 and T1, respectively, and were visualized by PAGE in dilutions of up to 10⁻⁸ and 10⁻⁹ for T0 and T1, respectively (figure 1). Clearance of *T. cruzi* was observed at T2, and its presence was confirmed only by weakly positive hybridization at most concentrated dilutions in 2 of the 4 extractions of DNA recovered from previously boiled samples. In figures 1 and 2, faint lines can be observed in lanes 16, 17, and 19, which may correspond to extremely low concentrations of *T. cruzi* DNA. However, after hybridization of the PCR products, the amplification of parasite DNA was weakly seen in undiluted CSF.
obtained at T0 and diluted from 10^{-2} to 10^{-8}, respectively, demonstrating amplification of 330-bp band up to 10^{-6} dilution corresponding to DNA of T. cruzi. Lanes 8–15, DNA at T1 diluted 10^{-1} to 10^{-8}, respectively, with amplified products visualized up to 10^{-7} dilution. Lanes 16–19, DNA at T2, pure and diluted 10^{-1} to 10^{-3}, respectively, with weak and doubtful T. cruzi kinetoplast DNA amplification. MW, molecular weight.

Figure 1. Polyacrylamide gel (6%) showing PCR amplification of Trypanosoma cruzi in CSF over time. Presence and subsequent elimination of parasite during treatment can be observed. DNA samples were obtained before (T0) and during treatment at 7 (T1) and 22 days (T2). Lanes 1–7, DNA obtained at T0 and diluted from 10^{-2} to 10^{-8}, respectively, demonstrating amplification of 330-bp band up to 10^{-6} dilution corresponding to DNA of T. cruzi. Lanes 8–15, DNA at T1 diluted 10^{-1} to 10^{-8}, respectively, with amplified products visualized up to 10^{-7} dilution. Lanes 16–19, DNA at T2, pure and diluted 10^{-1} to 10^{-3}, respectively, with weak and doubtful T. cruzi kinetoplast DNA amplification. MW, molecular weight.

Figure 2. Slot-blot demonstrating hybridization of PCR-amplified products in CSF at 7 (T1) and 22 days (T2) of treatment. Slots 1 and 2, positive controls. Slots 21 and 22, negative controls. Slots 3–11, PCR of DNA from CSF at T1, pure and diluted from 10^{-1} to 10^{-6}, respectively, demonstrating presence of Trypanosoma cruzi DNA up to 10^{-6} dilution. Slots 12–20, PCR of DNA from CSF at T2, pure and diluted from 10^{-1} to 10^{-8}, respectively, weakly showing presence of T. cruzi DNA in most concentrated samples.

(lane 16; slot 12) and in 10^{-1} (lane 17; slot 13) and 10^{-4} dilutions (not shown on polyacrylamide gel; slot 16). The presence of residual T. cruzi DNA was not detected at other dilutions (10^{-3} to 10^{-8}) on the polyacrylamide gel (data not shown) and hybridization was negative (slots 17–20). The product visualized in lane 19 corresponds to nonspecific amplification demonstrated by the negative hybridization (slot 15). Although successive dilutions of the DNA sample were done, amplification of T. cruzi DNA, although weak, was observed in some aliquots and not in others. This may have occurred as a result of the lack of targets for PCR, which were not distributed in an homogeneous manner in the diluted DNA sample. No inhibitors were detected in these samples, and the negative results were thought to indicate the absence of T. cruzi DNA. The DNA samples obtained from blood were diluted up to 10^{-4}, and PCR strongly amplified T. cruzi k-DNA in T1 and T2 as well as in T0 samples.

The genetic characterization of the hypervariable region of T. cruzi minicircle by use of LSSP-PCR revealed identical genetic signature profiles in the blood and CSF before treatment (T0) and 1 week later (T1), suggesting the presence of the same parasitic population. The genetic homogeneity of the parasite was also observed in the band profiles generated by the parasites isolated from culture (figure 3). The T. cruzi CL strain used as a control for LSSP-PCR presented its own band profile, which differed from that of the study (figure 3).

The T. cruzi population isolated from this patient (from samples of both blood and CSF) corresponded to the parasitic lineage 1 (T. cruzi II) associated with the domestic transmission cycle, with products amplified in the 125-bp region.

Discussion. Although regional endemic diseases are not classified among the infections associated with HIV or AIDS by the Centers for Disease Control and Prevention, the opportunistic character of Chagas’ disease [8, 10] is illustrated by the increased number of parasites present in both blood and CSF and the low number of CD4+ T lymphocytes (67 cells/mm³). The group of persons coinfected with HIV and T. cruzi is unknown, although data presented at scientific events indicate that this group is larger than the group of persons in whom chagasic infection is reactivated, which is only 6%–16.7% [10, 12]. Highly active antiretroviral therapy and the use of azolic derivatives in antifungal therapy probably contributes to the low rate of reactivation in coinfected persons.

Parasitemia seems to perform an important role in the reactivation of Chagas’ disease; it may precede the clinical manifestations or be detected later [11, 13, 25], being greater in patients with the clinical form of chagasic meningoencephalitis than in patients with the myocardial form [26]. The speed of detection of T. cruzi at the beginning of specific treatment may reduce its multiplication and impede dissemination of the infection to the CNS and heart [25]. In the cases of CNS invasion, clinical and tomographic similarities with neurotoxoplasmosis may make diagnosis more difficult [8, 27, 28], delaying the initiation of adequate therapy.

The drugs available for treatment of chagasic meningo-
Cephalitis are not always effective, and the outcome varies [7, 8, 12, 27, 29, 30]. Therapeutic control is based on the reduction and disappearance of the lesions (visualized by means of tomography), on the elimination of the parasite in CSF, and on the subsequent clinical improvement of the patient. Diagnostic techniques, such as xenodiagnosis and LIT medium culture, although specific, are slow and have low sensitivities. These techniques may give false-negative results in cases of low-level parasitism, making evaluation of the patient’s clinical state inconclusive and slow.

PCR is efficient for detecting low levels of blood parasitism in patients with chronic Chagas’ disease, demonstrating 1 parasite in 20 mL of blood [17] or as little as 0.1 fg of T. cruzi k-DNA [18]. The data we present show that, in the case of CNS invasion, PCR demonstrated an excellent capacity for detecting T. cruzi and controlling treatment, because it clearly demonstrated the presence and elimination of the parasite in the CSF. A positive PCR result in CSF suggests the presence of an intracellular T. cruzi cycle in the cerebrum and/or its recent destruction. The increased concentration of DNA in the CSF associated with the decrease of parasitism observed 1 week after the initiation of treatment (T1) seems to be a reflection of intense destruction of the parasites by the action of benznidazole. The use of PCR to monitor T. cruzi during treatment may be a rapid and safe indicator of the susceptibility of the parasite to the action of the drugs, allowing early changes in therapeutic conduct in cases of resistance. In this case, although treatment with benznidazole was initiated late, and although its tissue action was not evaluated, its capacity to suppress T. cruzi in the CSF and reduce parasitemia was significant, as evidenced by parasitological examinations and PCR results.

The continued detection of the parasite in blood at T2, at a time when CSF was essentially free of evidence of infection, may be attributed to the treatment, which, although not curing the infection [10], could be responsible for the presence of a degenerate nest of T. cruzi amastigotes or the absence of parasites during histological examination [11, 30]. The duration of persistence of T. cruzi DNA in the vertebrate host is still undetermined; some experimental evidence suggests that it is eliminated from the blood circulation within 48 h [31]. In this study, the weak amplification of residual DNA observed at T2 compared with T1 suggests that its clearance in human CSF may take at least 15 days.

The diversity of clinical manifestations of Chagas’ disease in humans has been attributed to the host’s immune response and to the genetic heterogeneity of the parasite, which may be formed by a multiclonal population with various biological profiles [32–34]. The preferential location of T. cruzi in the CNS is still unclear and may be associated with the presence of subpopulations with neurotropic characteristics that may be repressed by the immunologic response during the acute phase of infection and later reactivated during the immunosuppression of the host. Experimental evidence demonstrated that, after immunosuppression in mice in the chronic phase of chagasic infection, new subpopulations of T. cruzi with different genetic properties may emerge, with subsequent development of meningoencephalitis [35].

Other published data, however, indicate that immunosuppression resulting from HIV infection does not lead to the establishment of new T. cruzi genotypes, because T. cruzi stocks isolated from these patients were closely related to clonal genotypes previously identified (genotypes 30 or 32) in 89% and 94% of the stocks isolated from HIV-positive and HIV-negative patients, respectively [26].

In this investigation, the genetic characterization of T. cruzi k-DNA directly in CSF and blood of the patient by use of LSSP-PCR permitted a true analysis of the parasitic population involved in the pathological process. The genetic signature profile detected by the LSSP-PCR technique may correspond to ≥1 subpopulations of the parasite, but the variability in the k-DNA sequence of T. cruzi studied does not provide any data relative to the clonal or multiclonal structure, which could be evaluated by the analysis of polymorphic microsatellite loci [33]. The fact that the genetic signature profiles found in the population in the CNS and the blood were identical suggests that, in this case at least, immunosuppression did not induce exacerbation of different subpopulations. The homogeneity of the band profiles generated by LSSP-PCR before and 1 week after treatment also indicated that, at least during this period, benznidazole did not induce selection of parasitic subpopulations. The same was seen in the parasites isolated from culture medium. The possibility that the strain studied corresponds to a monoclonal population, however, should not be excluded and would explain the identity of LSSP-PCR profiles in all of the situations analyzed.

Characterization of the T. cruzi sequence of the 24Sα rRNA
gene allowed *T. cruzi* to be divided into 2 groups or lineages corresponding to the domestic cycle (lineage 1 [*T. cruzi* II]) and to the sylvatic cycle (lineage 2 [*T. cruzi* I]), the biological significance of which is under discussion. Experimentally, only *T. cruzi* genotype 20 appears to be associated with CNS invasion [16]. This genotyping belong to lineage 2 [36], and its multiplication is easily controlled by the host's immune system [37]. The *T. cruzi* strain evaluated in the blood and the CSF demonstrated that it was lineage 1 (*T. cruzi* II), which is predominant in infections in humans, and which is also common in the area of endemicity from which the patient originated [38]. It has been suggested that the strains of *T. cruzi* associated with invasion of the CNS may be genetically similar to the CL strain [39]. However, although the strain studied and the CL strain both belong to lineage 1, the patterns of their genetic signatures determined by LSSP-PCR were different, indicating that their k-DNA possesses distinct sequences. These data reinforce the necessity for further studies characterizing the subpopulations of *T. cruzi* for definition of the genetic profiles associated with the differentiated tropisms [34], such as neurotropism.

Adequate monitoring of *T. cruzi* in patients who carry sequences with the genetic potential to invade the CNS may possibly prevent future cases of chagasic disease reactivation in patients coinfected with HIV. PCR is a sensitive, specific, and fast technique for the detection of *T. cruzi* in the CSF; it may be an effective tool for the early diagnosis and monitoring of treatment of these patients and should be further investigated.

References