

Transfusional Chagas Disease: Parasitological and Serological Monitoring of an Infected Recipient and Blood Donor

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Chagas disease is endemic to Latin America, but human migration is extending its distribution. This report describes the parasitological and serological course of disease in a Spanish patient fatally infected via a blood product transfusion, as well as the monitoring of the donor. Before undergoing immunosuppression, multitransfused patients should be screened for anti-*Trypanosoma cruzi* antibodies.

Chagas disease, or American trypanosomiasis, is endemic to Latin America. However, the recent changes in human patterns of migration have prompted the appearance of cases in areas where the vector of the disease is not found [1, 2]. The natural progress of infection involves an acute and a chronic phase. In areas of endemicity both forms are seen, whereas in nonendemic areas, the great majority of infections are diagnosed in the chronic phase, although 70% of infected persons remain asymptomatic. Despite technological advances, there is no reference standard laboratory technique for diagnosing Chagas disease [3]. In the acute phase, parasitological diagnostic methods are the most reliable. However, in the chronic phase there may be little or no parasitemia, and diagnosis is made mainly on the basis of results of tests for anti-*Trypanosoma cruzi* antibodies. In recent years, Spain has become one of the favorite destination countries for South American emigrants. These citizens achieve a good degree of social integration in Spain, and

they often voluntarily and altruistically support blood donation programs. Thus, since 2005, Spanish blood donation legislation has required donors from Latin America to be serologically screened for anti-*T. cruzi* antibodies (Royal Decree 1088/2005) [4]. The present work describes the retrospective laboratory evaluation of a Spanish patient with leukemia who died of Chagas disease contracted via a transfusion with contaminated blood, the retrospective study to identify the source of infection, and the monitoring of the donor.

Methods and materials. Anti-*T. cruzi* antibodies were sought in serum samples collected at different times before the patient's death; these samples were stored at -80°C in the serum library of the Centro Nacional de Microbiología (National Microbiology Center [Madrid]). Parasite DNA was also sought in these samples, in CSF (also collected before death), and in lung, kidney, and liver necropsy samples.

For the retrospective study, serum samples of 176 donors whose blood derivatives had been transfused into the patient were examined. Of these, 168 lived in Madrid (159 were of Spanish origin, 1 was Brazilian, 1 was Ecuadorian, 2 were Colombian, 3 were French, 1 was Polish, and 1 was German), 5 lived in Albacete (southeastern Spain), and 3 lived in Jaén (southern Spain). Samples belonging to all of the Madrid donors were preserved at the serum library of the Centro de Transfusión de Madrid (Madrid Transfusion Center); new samples were collected from the donors living in Albacete and Jaén once they had been traced. Serum and blood samples were collected from the infected blood donor to confirm the results of the retrospective study and to monitor the development of the infection after treatment.

Anti-*T. cruzi* antibodies were detected by the indirect immunofluorescent antibody test (IFAT) and by ELISA with modifications introduced by the Department of Parasitology at the Centro Nacional de Microbiología [5, 6]. *T. cruzi* DNA was detected by PCR with use of oligonucleotides 121–122 and Tcz1–Tcz2, which amplify the variable region of the kinetoplast DNA minicircle (330 bp) and a repetitive sequence of satellite DNA (195 bp), respectively [7, 8]. All assays were performed in duplicate with negative and positive controls.

Results. The Spanish patient was a 25-year-old man who had a history of leukemia [9] that eventually required a cord blood transplant; he received blood derivatives from at least 176 persons who donated blood at different transfusion centers. In January 2005, 45 days after infection onset, the patient was examined for fever of unknown origin. None of the infectious agents that commonly cause this problem in this kind of patient

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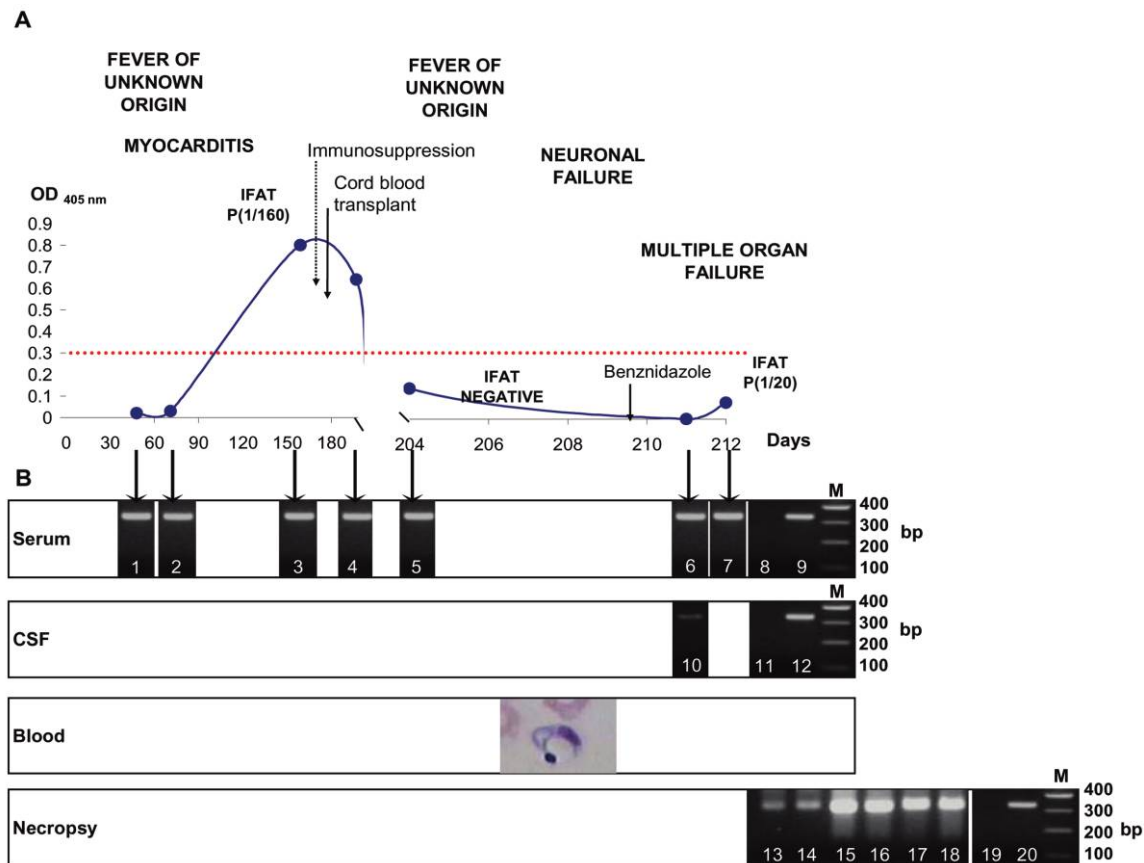


Figure 1. Parasitological and serological changes according to the clinical status of the patient. The day the patient received the platelet concentrate was defined as day 0. *A*, Changes in anti-*Trypanosoma cruzi* antibody levels according to indirect immunofluorescent antibody test (IFAT) and ELISA. The last serum dilution with a positive (P) reaction is shown. *B*, Presence of parasites as determined by microscopy and PCR. Lanes 8, 11, and 19, Negative controls. Lanes 9, 12, and 20, Positive controls. Lanes 13–14, 15–16, and 17–18, Duplicate samples of *T. cruzi* DNA amplified from kidney, liver, and lung tissues, respectively. The PCR results obtained using the oligonucleotides 121–122 confirmed those obtained with Tcz1–Tcz2. Dotted line, Threshold; OD, optical density.

(lymphotropic viruses, exanthema-causing viruses, adenoviruses, influenza virus, *Mycoplasma pneumoniae*, or *Toxoplasma gondii*, among others) were detected. After treatment with itraconazole, the symptoms receded, and the patient was assessed and treated in preparation for cord blood transplantation as described by Forés et al. [9]. In the first week of July 2005 (day 211 after infection onset), the Department of Parasitology at the Centro Nacional de Microbiología received several serum and CSF samples obtained from the patient, as well as the supernatants of cell cultures used in the identification of flagellates by microscopy and in diagnostic tests. Microscopy revealed the presence of trypomastigotes, and PCR identified DNA of *T. cruzi*, indicating infection by this pathogen. Tests for anti-*T. cruzi* antibodies, however, yielded negative results.

The patient died of multiorgan failure (day 212 after infection onset), and a retrospective evaluation was undertaken to determine the source of infection. Patient serum samples that were sent to the Centro Nacional de Microbiología for the

diagnosis of problems other than Chagas disease and that were preserved at our center's serum library were analyzed by IFAT, ELISA, and PCR (figure 1). PCR showed *T. cruzi* to have first appeared in the patient's serum 48 days after he received a transfusion of platelets. IFAT and ELISA confirmed positive seroconversion on day 159 after infection onset, followed by a negative seroconversion on day 204 after infection onset.

At the same time, the donors whose blood products had been given to the patient were screened for anti-*T. cruzi* antibodies (figure 2A). This analysis ruled out all of the donors from Albacete and Jaén and 167 of the donors from Madrid as potential sources of infection. IFAT and ELISA yielded positive results for the remaining Madrid-based donor. This person made a blood donation at the beginning of December 2004 (figure 2B); the patient received a concentrate of platelets prepared from this blood (day 0).

The donor was a 58-year-old woman originally from Alto Parnaíba, in the Brazilian state of Maranhão. She was asked to

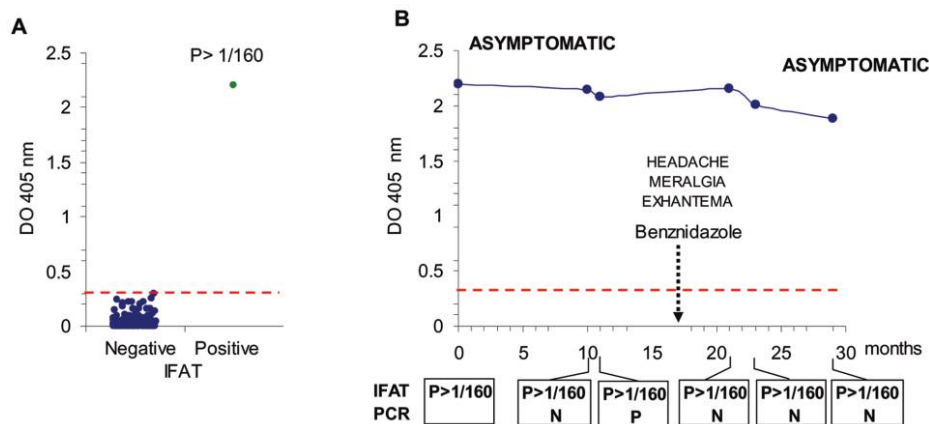


Figure 2. A, Determination of anti-*Trypanosoma cruzi* antibodies by indirect immunofluorescent antibody test (IFAT) and ELISA in serum of the different blood donors. The last serum dilution with a positive (P) reaction is shown. B, Serological and PCR monitoring of the infected donor. The month in which the infected donor made a blood donation was defined as month 0. Dotted line, Threshold. N, negative; OD, optical density.

attend an appointment to confirm the results obtained in the retrospective investigation. At that time, no parasites were detected in her blood by PCR. She was then referred to the Tropical Diseases Unit at the Hospital Carlos III in Madrid, where she underwent a clinical examination, chest radiography, electrocardiography, and echocardiography, all of which yielded normal results. No other signs or symptoms of interest were noted except for constipation, which the donor had experienced for some 8 years (defecation once every 2–3 days). On this occasion (1 month after the first appointment), however, PCR did detect parasites in the blood. In March 2006, treatment with benznidazole (6 mg/kg/day) was begun, but this was suspended after 24 days because of the appearance of intense headaches, meralgia paresthetica of the femorocutaneous nerve, and generalized macular exanthema. No hematologic toxicity was recorded. Following this treatment, test results for blood parasites remained negative, although anti-*T. cruzi* antibodies remained detectable (figure 2B).

Discussion. Figure 1 shows that anti-*T. cruzi* antibodies were detectable in the patient only before the start of the immunosuppressive protocol associated with the cord blood transplant (day 159 after infection onset). In the absence of an immune response, the parasites crossed the blood-brain barrier and infected the nervous system. This was confirmed by the presence of *T. cruzi* DNA in the CSF sample. Given the general condition of the patient, treatment with benznidazole had no immediate effect on the parasite load, although IFAT detected a slight increase in the antibody titer (1/20).

The detection of *T. cruzi* in the necropsy samples agrees with the systemic distribution of the parasite and the multiorgan failure that caused the patient's death. The kinetics of the antibody titer can be explained in terms of an acute, recently acquired infection. The detection of *T. cruzi* by PCR since Jan-

uary 2005 (day 48 after infection onset) agrees with the date when the patient received the infected blood products. Thus, the results of the parasitological and serological investigations agree with the patient's clinical signs and symptoms and suggest that he was in the acute phase of Chagas disease. Acute transfusional Chagas disease can last from 1 to 6 months after the entry of the parasite [3].

The discrepant PCR results (1 positive and 1 negative) obtained for the infected donor before benznidazole treatment was begun agree with the low-level parasitemia typical of the chronic phase of *T. cruzi* infection [10]. These results could also be because the first analysis involved a 5-mL blood sample and the second a 10-mL sample. When blood parasite concentrations are low, detection is more likely in larger blood volumes [11]. Similarly, at blood donation units, collecting as much as 450 mL of blood from donors increases the risk of contamination with small numbers of parasites.

Although, for successful blood culturing and artificial xenodiagnosis, it is recommended that blood samples be processed within 4 h of collection to ensure parasite viability [12]; in the present case, the parasites remained viable over the entire platelet conservation period, because the maintenance temperature (22°C; range, 20°C–24°C) is close to that used for culturing *T. cruzi* (25°C–27°C). The recipient's immunodepression caused by his leukemia and the immunosuppression induced before cord blood transplantation appear to have been of maximum importance in the development of the infection, because the parasite caused no appreciable symptoms in the donor. This highlights the role of the host immune system in protection from and the development of infection. In immunodepressed patients, infection may be severe and have fatal consequences. It is therefore recommended that higher-risk organ donors be screened for anti-*T. cruzi* antibodies, as should

multitransfused candidates for transplantation—irrespective of their origin—if they are to undergo immunosuppression protocols.

It should be stressed that before October 2005, Spanish blood donation legislation permanently excluded donors with Chagas disease. It did not, however, contemplate the use of a reliable screening test for the detection of healthy *T. cruzi* carriers. In the present case, the donor did not know of her trypanosome infection status, and no risks were detected during the pre-donation assessment interview. Her blood donation was therefore accepted in December 2004. In contrast, the current legislation (October 2005) outlines new technical requirements for blood donation [4] and establishes the use of a *T. cruzi* diagnostic assay to assess the eligibility of donors from areas where Chagas disease is endemic, as well as those with risk factors for infection. Under this legislation, the present donor would have been excluded.

In Spain, the supply of blood is a permanent problem, and the Latin American population—~1.5 million residents—has already become an important source of potential donors. A preliminary *T. cruzi* seroprevalence survey of immigrants from areas of endemicity returned positive estimates of close to 1% [13]. Because blood transfusion is the main route for *T. cruzi* transmission in Spain, the new legislation guarantees the quality of blood and blood component transfusions for recipients and allows the inclusion of immigrants from the Americas in the pool of potential blood donors.

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Potential conflicts of interest. All authors: no conflicts.

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