Reactivation of Chagas Disease in a Patient With Follicular Lymphoma Diagnosed by Means of Quantitative Real-Time Polymerase Chain Reaction

Maria I. Garzón,[1] Ariel G. Sánchez,[2] Maria C. Goy,[2] Teresita Alvarellos,[2] Abel H. Zarate,[1] Ana L. Basquiera,[3] Juan J. Garcia,[3] and Juan P. Caeiro[1]

[1]Infectious Diseases Section; [2]Molecular Biology Laboratory, and [3]Hematology Section, Hospital Privado Centro Médico de Córdoba, Argentina

We report a case of Chagas disease reactivation in a patient with stage IIb follicular lymphoma in the cecum. He was admitted to the hospital with neutropenia and fever. He had a history of right hemicolectomy 6 months earlier and had received the sixth cycle of chemotherapy with cyclophosphamide/doxorubicin/vincristine/prednisone/rituximab. Blood and urine cultures were negative, but the fever persisted. Reactivation of Chagas disease was confirmed by means of quantitative real-time polymerase chain reaction (qRT-PCR). Parasitic load was 577 950 parasite equivalents/mL. The patient began treatment with benznidazole 5 mg/k per day every 12 hours. After 1 month, the qRT-PCR control was undetectable. The patient completed 60 days of treatment and is currently asymptomatic. *Trypanosoma cruzi* qRT-PCR may become a useful diagnostic method for reactivation of Chagas disease.

Keywords. chagas disease; immunocompromise; qRT-PCR; reactivation.

Chagas disease is one of the most prevalent parasitic infections: it has extended from Latin America to nonendemic areas such as the United States, Canada, and Europe [1].

There are an estimated 8 million people infected worldwide, predominantly in endemic areas of South and Central America, and 20% to 30% of them develop potentially life-threatening symptomatic diseases [2]. In Argentina, there are an estimated 2 000 000 people infected with Chagas desease, and approximately 600 000 could present clinical anomalies compatible with chronic Chagas disease [3].

Reactivation of Chagas disease has been reported in immunocompromised patients, such as solid organ recipients, patients with hematologic diseases with or without bone marrow transplant, and people with acquired immune deficiency syndrome [4]. In these patients, reactivation is usually associated to severe clinical manifestations, central nervous system involvement, chagomas, meningoencephalitis, and myocarditis. Patients with moderate immunosuppression and latent Chagas disease are usually asymptomatic, and parasitemia increases before the symptoms appear.

Chagas disease may reactivate in persons with lymphoma receiving chemotherapy [5]. The reactivation of Chagas disease is defined as parasitemia detectable with different techniques such as direct parasitological exam or polymerase chain reaction (PCR), even in the absence of symptoms [6]. For Chagas reactivation, early therapy with benznidazole has demonstrated to be efficacious, although there is no international consensus on how to treat these patients [7]. Our group has used PCR to diagnose the reactivation of Chagas disease on heart transplant recipients [8].

CLINICAL CASE

A 60 year-old male with history of stage IIb follicular lymphoma in the cecum was admitted to our institution for febrile neutropenia and asthenia. He underwent right hemicolectomy 6 months earlier and received the sixth cycle of chemotherapy with cyclophosphamide/doxorubicin/vincristine/prednisone/rituximab 1 week prior. Upon admission, he received empirical treatment with piperacillin-tazobactam after blood cultures were drawn, but the fever persisted. A thoracic computed tomography scan was performed without significant findings. The patient had history of positive Chagas serology 5 years earlier. On the eighth day of hospitalization, he remained with fever and asthenia but without positive cultures or response to antibiotics. New blood cultures for bacteria and fungi were obtained. A blood smear searching for parasites and Chagas quantitative real-time PCR (qRT-PCR) was carried out. Parasites were not observed in Giemsa-stained blood smear. The qRT-PCR for Chagas was 577 950 parasite equivalents (Par Eq)/mL (Figure 1A). Nucleic acids were isolated from 400 μL

Received 26 December 2014; accepted 28 April 2015.

Correspondence: Juan P. Caeiro, MD, Servicio de Infectología, Hospital Privado Centro Médico de Córdoba, Naciones Unidas 346, Córdoba 5016, Argentina (jpcaeiro3@gmail.com).

Open Forum Infectious Diseases

DOI: 10.1093/ofid/ofv060

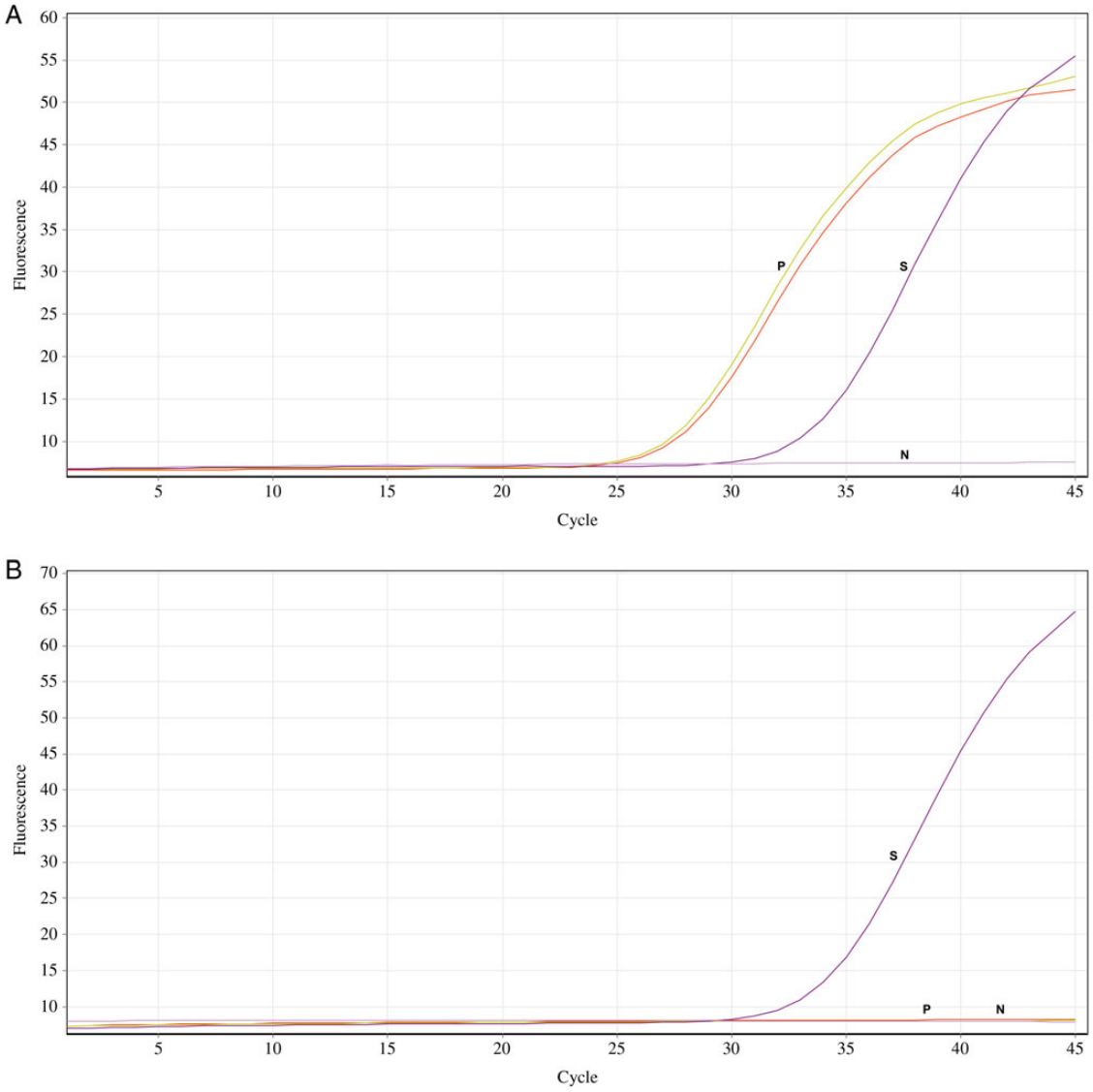

Figure 1. (A) Amplification curve of *Trypanosoma cruzi* before treatment. Cycle threshold: 23 for patient and 29 for standard. (B) Amplification curve of *T cruzi* after treatment. Cycle threshold: 28.9 for standard. Abbreviations: N, no template control (NTC); P, patient, duplicate samples; S, standard (4500 parasite equivalents/mL).

peripheral blood through MagNA Pure Compact Nucleic Acid Isolation kit 1 (Roche) and eluted in 100 µL. Quantitative real-time PCR was performed according to Piron [5] with modifications, using 2X Universal KAPA Master mix (Kapabiosystems), 10 µM TaqMan probe (Applied Biosystems), 750 nM of each primer, and 5 µL DNA sample, on a real-time thermocycler (Rotor-gene 6000; Corbett Research), with the aim of amplifying a nuclear satellite fragment of 166 base pairs from repeated region of parasitic satellite DNA. To generate a standard curve, DNA was extracted from blood sample spiked with Y strain trypomastigotes culture (Ref. number Medline: 97179491) as described previously [6]. Parasite load was determined using an absolute quantification method with a linear range of 50–1 000 000 Par Eq/mL. To validate this procedure, 1 standard of quantification (4500 Par Eq/mL), which represents a point in the standard curve accomplished earlier, and nontemplate control were included in the run.

The threshold used for quantification analysis was 0.0246 for all runs in this work (Figure 2).

The blood smear and prueba cutánea de tuberculina, derivado proteico purificado de tuberculina (PPD) were negative. The patient was placed on benznidazole 5 mg/k per day every 12 hours with good tolerance to the medication. After 1 month, parasitemia level decreased to undetectable (Figure 1B). The patient completed 60 days of treatment and is currently asymptomatic.

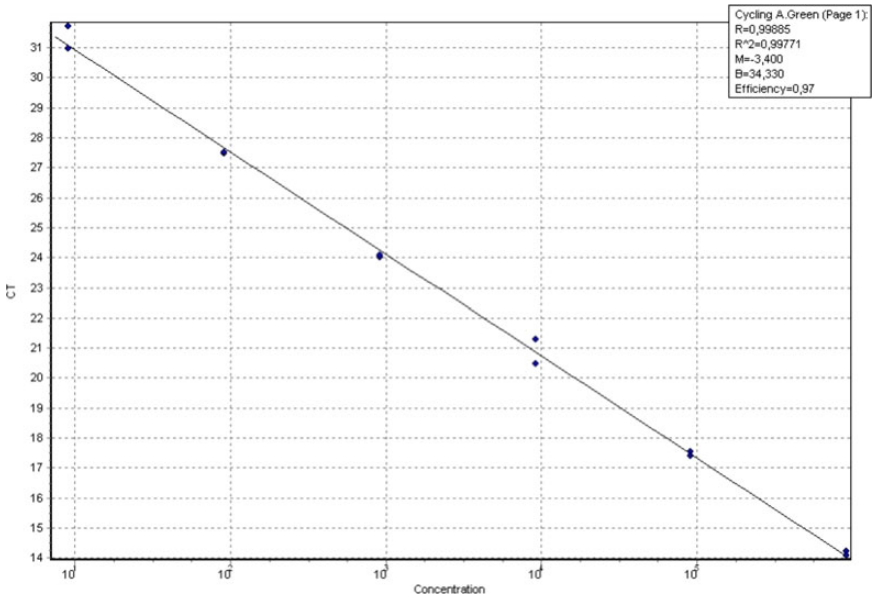

Figure 2. Standard amplification curve made by serial dilutions of DNA from blood spiked with *Trypanosoma cruzi* (threshold: 0.0246; R2: 0.997).

DISCUSSION

The high parasitic load in our patient could be considered as an indicator of reactivation. It has recently been shown that the qRT-PCR technique can differentiate reactivated patients versus chronic infected patients with human immunodeficiency virus/*Trypanosoma cruzi* [9]. It has been observed that Chagas qRT-PCR is more sensitive than direct conventional methods and allows earlier detection of the parasite, even before the appearance of symptoms associated with reactivation [10]. In this case, the patient received 60 days of therapy and after 1 month of treatment, he had a Chagas qRT-PCR undetectable. There are no guidelines for Chagas disease reactivation in patients with lymphoma. Some experts recommend monitoring of reactivation and treat accordingly [11].

Early therapy with benznidazole or nifurtimox must be considered immediately after the finding of positive results with direct parasitological exams, increase of parasitic load, or reactivation of symptoms.

CONCLUSIONS

The main objective of our study was to demonstrate the importance of the reactivation of Chagas disease in patients with neoplastic diseases and chronic Chagas, which require constant and intense immunosuppressive therapy. Early detection of parasitemia through molecular techniques could assist in the control of immunocompromised patients with Chagas reactivation. Preemptive therapy of Chagas disease should be accomplished. Although international consensus has not been reached on the care of these patients, in case of reactivation, early therapy with benznidazole may be effective.

Acknowledgments

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Schmunis GA, Yadon ZE. Chagas disease: a Latin American health problem becoming a world health problem. Acta Trop **2010**; 115:14–21.
2. Bern C, Kjos S, Yabsley MJ, Montgomery SP. *Trypanosoma cruzi* and Chagas' disease in the United States. Clin Microbiol Rev **2011**; 24:655–81.
3. Riarte A, Luna C, Sabatiello R, et al. Chagas' disease in patients with kidney transplants: 7 years of experience, 1989–1996. Clin Infect Dis **1999**; 29:561–7.
4. Altclas J, Sinagra A, Dictar M, et al. Chagas disease in bone marrow transplantation: an approach to preemptive therapy. Bone Marrow Transplant **2005**; 36:123–9.
5. Rezende RE, Lescano MA, Ramalho LN, et al. Reactivation of Chagas' disease in a patient with non-Hodgkin's lymphoma: gastric, oesophageal and laryngeal involvement. Trans R Soc Trop Med Hyg **2006**; 100: 74–8.
6. Pinazo MJ, Espinosa G, Cortes-Lletget C, et al. Immunosuppression and Chagas disease: a management challenge. PLoS Negl Trop Dis **2013**; 7:e1965.
7. Bern C. Chagas disease in the immunosuppressed host. Curr Opin Infect Dis **2012**; 25:450–7.
8. Maldonado C, Albano S, Vettorazzi L, et al. Using polymerase chain reaction in early diagnosis of re-activated *Trypanosoma cruzi* infection after heart transplantation. J Heart Lung Transplant **2004**; 23:1345–8.
9. de Freitas VL, da Silva SC, Sartori AM, et al. Real-time PCR in HIV/*Trypanosoma cruzi* coinfection with and without Chagas disease reactivation: association with HIV viral load and $CD4^+$ level. PLoS Negl Trop Dis **2011**; 5:e1277.
10. Pavía PX, Roa NL, Uribe AM, Puerta CJ. [Using S35-S36 and TcH2AF-R primer-based PCR tests to follow-up a Chagaś disease patient who had undergone a heart transplant]. *Biomédica* **2011**; 31:178–84.
11. Lattes R, Lasala MB. Chagas disease in the immunosuppressed patient. Clin Microbiol Rev **2014**; 20:300–9.