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Screening for Chagas' disease in HIV-positive immigrants from endemic areas

A. RODRÍGUEZ-GUARDADO[1]*, V. ASENSI ALVAREZ[1], M. RODRÍGUEZ PEREZ[2], P. MEJUTO ALVAREZ[2], M. FLORES-CHAVEZ[3], P. ALONSO GONZÁLEZ[2] AND J. A. CARTÓN SÁNCHEZ[1]

[1] *Infectious Diseases Unit,* [2] *Microbiology Unit, Hospital Universitario Central de Asturias, Oviedo, Spain*
[3] *Parasitology Department, Centro Nacional de Microbiología* (*National Microbiology Centre*), *Instituto de Salud Carlos III, Madrid, Spain*

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SUMMARY

Chagas' disease is an opportunistic infection in the setting of HIV/AIDS. The arrival of HIV-positive immigrants from endemic areas to non-endemic countries makes possible the detection of Chagas' disease in this group of patients. We describe the results of a screening programme conducted in the HIV-positive immigrant population arriving from endemic areas who attended the Tropical Medicine Unit of Hospital Universitario Central of Asturias during 2008. We determined anti-*T. cruzi* antibodies in all HIV patients arriving from endemic areas who were followed up. The ID-Chagas antibody test was used as a screening assay. The positive cases were confirmed with ELISA, IFAT and PCR. We analysed 19 HIV-positive immigrants, of which two (10·5%) had a positive antibody test for Chagas' disease confirmed. PCR was positive in both cases. There was no difference between the co-infected and the non-co-infected patients with respect to race, place of birth and residence, CD4+ cell count, and HIV viral load count. Direct microscopic examination of blood was negative in both positive cases. The positive patients were a man from Bolivia and woman from Paraguay. The overlap of HIV and *T. cruzi* infection occurs not only in endemic areas but also in non-endemic areas of North America and Europe where the diagnosis may be even more difficult due to low diagnostic suspicion. The implementation of screening programmes in this population group is needed for the early diagnostic of Chagas' disease.

Key words: Chagas' disease, epidemiology, HIV infection, opportunistic infections, *Trypanosoma cruzi*.

INTRODUCTION

Human infection with the protozoa *Trypanosoma cruzi* extends through North, Central, and South America affecting 21 countries. Estimates suggest that 8–9 million people are chronically infected with *T. cruzi* and approximately 90 million individuals remain at risk of contracting the infection [1–4]. On the

* Author for correspondence: Dr A. Rodríguez-Guardado, Infectious Diseases Unit, Hospital Central de Asturias, C/Celestino Villamil s/n, 33006, Oviedo, Spain. (Email: azucenarodriguez@telecable.es)
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other hand, according to UNAIDS estimates for South America, approximately 1·7 million people (range 1·5–2·1 million) are infected with human immunodeficiency virus (HIV) which makes co-infection with both diseases increasingly likely [5]. Reactivation of Chagas' disease is characterized by acute and severe clinical manifestations, mainly meningoencephalitis or myocarditis, and high *T. cruzi* parasitaemia, which has been reported in patients with HIV [6–13].

The arrival of individuals from Chagas-endemic areas to non-endemic countries due to immigration makes the occurrence of this disease possible in patients from non-endemic areas where diagnosis of this disease may be difficult. Due to this fact the need for technical screening for the disease in this population group has been postulated [6–8].

This study describes the results of a screening programme conducted in the HIV-positive immigrant population from endemic areas arriving in Asturias, an area in northern Spain where 3·8% of the population is foreign-born.

MATERIAL AND METHODS

During 2008 anti-*T. cruzi* antibodies were determined in all HIV patients from endemic areas, with infection confirmed by Western blot, that were followed up at the Infectious Disease Unit of Hospital Universitario Central of Asturias, a reference centre with 1200 beds. A questionnaire was given to all patients, which included items addressing history of Chagas' disease, any previous transplants or transfusions, and characteristics of the place where they lived.

The ID-Chagas antibody test [particle gel immunoassay (PaGIA), DiaMed, Switzerland] was used as a screening assay: red-coloured polymer particles are sensitized with three different peptides representing antigenic sequences of *T. cruzi*. The technique was performed according to the manufacter's instructions. Positive cases were confirmed with a second ELISA (Ortho-Clinical Diagnostics, USA). The Ortho *T. cruzi* ELISA test system is an enzyme-linked immunosorbant assay for the qualitative detection of antibodies to *T. cruzi*. The assay utilizes microwells coated with a whole-cell lysate containing *T. cruzi* antigens as the solid phase [14]. All samples testing positive by any technique were sent to the National Microbiology Centre (Instituto Carlos III, Spain) to confirm the result by determination of anti-*T. cruzi* antibodies by indirect immunofluorescent antibody test (IFAT) and by polymerase chain reaction (PCR). *T. cruzi* DNA was detected by PCR with the use of oligonucleotides 121–122 and Tcz1–Tcz2, which amplified the variable region of the kinetoplast DNA minicircle (330 bp) and a repetitive sequence of satellite DNA (195 bp), respectively [15]. All assays were performed in duplicate with negative and positive controls. Total parasitaemia in blood was determined in positive patients through the Strout method. Five millilitres of total blood initially spun at 160 rpm was used in order to remove the parasite from the red cells. A second centrifugation was performed at 350 rpm in order to obtain sediment used to identify the parasite. This sediment was dyed with the classic May–Grunwald–Giemsa or trichrome stain.

Chagas' disease was defined as a positive result for two different serological techniques. In all confirmed cases a protocol that included a clinical-epidemiological evaluation, chest X-ray, EKG, oesophagogastroscopy, barium enema, cranial computed tomography, and echocardiography was applied. Routine tests for determination of liver enzyme levels, CD4+ cell counts, and HIV load were performed simultaneously with serological studies.

Statistical analyses

Continuous values were expressed as the mean and compared using Student's t test or Mann–Whitney U test. Categorical values were expressed as absolute and relative frequencies and were compared using Fisher's exact test or χ^2 test. A P value $<0{\cdot}05$ was considered as statistically significant.

Ethical considerations

The patients gave informed consent for the study and were assessed and treated in accordance with the standard protocols in our department.

RESULTS

During the study period, 19 HIV-positive immigrants were screened [12 males (63%), mean age 36 years, range 28–48]. The countries of origin were: Brazil and Ecuador [five cases (26%) each], Colombia [four cases (21%)], Paraguay, Uruguay, Argentina, Dominican Republic, and Bolivia [one case (5·2%) each)]. The average time of residence in Spain was 1335 days. None of the patients presented an indeterminate test. Two patients (10·5%) had a positive antibody test for

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Chagas' disease, which was confirmed in all cases. PCR was positive in both cases. There was no difference between the co-infected and the non-co-infected patients with respect to race, place of birth and residence, CD4+ cell count, and HIV viral load count. Direct microscopic examination of blood was negative in both positive cases. Prior to their arrival in Spain the two positive patients lived in rural areas of Latin American in dwellings covered with a straw roof where the reproduction of triatomine bugs is possible. One case was a man from Bolivia who had lived in Spain for 365 days prior to diagnosis. He was on highly active antiretroviral treatment (HAART) with 348 CD4+ cells/mm^3 and HIV-1 RNA <10 copies/ml. The second case was a woman from Paraguay who had lived in Spain for 487 days prior to diagnosis of Chagas' disease. She was on HAART with CD4 of 348 and 456 CD4+ cells/mm^3 and with HIV-1 RNA <10 copies/ml. Both patients were asymptomatic and had normal additional tests for Chagas' disease. All patients were treated with benznidazole (5 mg/kg per day for 90 days) with good tolerance. No evidence of hepatic or haematological toxicity was found. The PCR was negative 3 months after treatment and remained negative throughout the follow-up. The mean follow-up was 465 days.

DISCUSSION

Chagas' disease is caused by a protozoan parasite, *T. cruzi*, which is transmitted to humans through the faeces of infected bloodsucking insects in endemic areas of Latin America, or occasionally by non-vectorial mechanisms. With regard to these transmission mechanisms there are parallels with HIV infection. Both diseases may be transmitted through blood transfusions and contaminated blood products [7]. Cases of vertical, transplacental infection of *T. cruzi* are seen in between 0·5% and 10% of the children of chagasic mothers according the region studied [3]. Other less probable ways of transmission include oral transmission due to contaminated drinks or uncooked meat, accidental contamination in the laboratory, and the potential transmission linked with organ transplants [8]. Several studies indicate the growing importance of intravenous drug use, where drug users share contaminated needles and syringes, as a mode of Chagas' infection transmission. The prevalence of co-infection is significantly higher in this group [9].

Migration has created a situation where many people infected by the parasite currently live in non-endemic areas where diagnosis may be difficult. The USA estimates that between 100 000 and 675 000 people may be infected as a result of the mass migration from Latin American countries. Spain is the European country with the largest number of immigrants from Latin America [10]. According to the Spanish Statistical Institute, 5 220 577 immigrants resided in Spain at the end of 2007 [11]. Of this number, 1 761 317 came from the Americas, the most frequent countries of origin being: Ecuador (420 110), Colombia (280 705), Bolivia (239 942), Argentina (145 315), Peru (120 272), Brazil (115 390), the Dominican Republic (76 954), Paraguay (66 710) and Venezuela (57 679). According to Eurostat [18] data from 2006, it was estimated that 25/1000 immigrants from endemic places could be infected. UNAIDS estimates the number of people currently living with HIV in South America as: 730 000 in Brazil, 170 000 in Colombia, 120 000 in Argentina, 26 000 in Ecuador, and 11 000 in Bolivia [5]. On other hand, according to data obtained from a Spanish cohort of patients recently diagnosed with HIV infection during 2004–2005, 16% of them came from Central and South America [12]. These data show that the occurrence of co-infection in this group of patients is likely to increase which necessitates questioning the existing protocols in order to deal with this new situation.

The co-existence Chagas' disease and HIV infection has been well documented in the literature [6, 13–19]. There are studies on the different side-effects of one disease compared to the other. In this way, *T. cruzi* parasitaemia may prove to be more frequently positive in these patients and may precede the clinical appearance of the disease. Therefore, the positivity of parasitaemia in these patients is associated with punctual increases of the HIV viral load [6, 13].

In patients with CD4+ lymphocyte counts <200 cells/μl the possibility of reactivation of chronic disease that appeared through acute neurological damage (75–90% of cases) or myocardic damage (30% of cases) has been widely described in the literature [14–18]. These methods show the reactivation of previous acute asymptomatic or oligosymptomatic disease in patients who presumably contracted the disease during their childhood when living in endemic areas.

Damage to the CNS may occur as space-occupying lesions indistinct from toxoplasmosis, or in acute meningoencephalitis with fever, cephalea, vomiting and focal neurological deficits. Since brain lesions are occasionally indistinct to those produced by

toxoplasmosis, simply starting an anti-toxoplasma treatment as usual may be wrong for those patients from areas with a high occurrence of *T. cruzi* infection, and will entail a delay in the diagnosis and implementation of treatment which, undoubtedly, makes the prognosis worse [14–17]. The occurrence of isolated myocarditis is common in these patients and used to be associated with CNS damage [14–17]. Several findings in necropsies suggest that a significant portion of co-infected patients in the apparently chronic stage have silent heart damage with parasite occurrence within the myocardium [19].

Mortality due to reactivation of Chagas' disease in patients with AIDS is very high. Most cases show a fatal evolution in 10–20 days after diagnosis. Early diagnosis and treatment can only improve survival if accompanied with HAART. Nevertheless, outside endemic areas, Chagas' infection is rarely suspected or investigated by the professionals who assist HIV-positive patients. Several studies in the literature from urban areas of endemic countries show a high percentage of HIV-positive patients followed up (45·3%) who have never been assessed for the disease [9].

Both positive patients in our study were in the asymptomatic latent stages of the disease although with positive PCR for *T. cruzi*. The fact that the majority of patients with Chagas' disease were in the latent stages makes this diagnosis more difficult outside endemic areas.

The treatment for Chagas' disease in Spain is benznidazole, administered for at least 60 days at a dose of 5–7 mg/kg per day, divided into three doses, although the treatment duration in HIV-positive patients has not been well defined [14, 20, 21]. The treatment has been especially problematic due to the likely interaction with antiretroviral drugs and the occurrence of side-effects (skin rash, peripheral neuropathy, granulocytopenia, impairment in test of liver function, etc.) in a similar manner to those described for the antiretrovirals, and may be increased by them. Given the necessity of setting up an early treatment in those patient groups, reactivation predicting factors have been attempted, e.g. positive parasitaemia detection in blood, which shows a higher predicting value (50%).

Nevertheless, the initial identification of these patients comprises serological strategies. For that reason anti-*T. cruzi* serology should be recommended systematically for any HIV-infected patient from an endemic area, having a diagnostic value for the occurrence of positive serology made against different antigens (indirect haemmaglutination, indirect immunofluorescence and ELISA). A specific treatment is recommended to all positive patients especially if they show a positive PCR for *T. cruzi* or parasitaemia detectable in blood by other methods even though they are asymptomatic. For that, a PCR technique generalization, only available in a reference laboratory currently, is needed in the daily clinic practice which enables improved diagnosis and early identification of patients susceptible to suffering a reactivation.

In conclusion, the epidemiological profile of AIDS and Chagas' disease over the past years might facilitate the approximation of both diseases, thereby increasing the possibility of reactivation of Chagas' disease in HIV patients. These cases have a high risk of not being correctly diagnosed, therefore adding to the seriousness and lethality of the disease. Serological tests for Chagas' disease, even when they are indeterminate, should be taken fully into account and the relevant parasitological tests must be performed.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Hotez PJ, *et al.*** The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. *PLoS Neglected Tropical Diseases* 2008; **24**: e300.
2. **Franco-Paredes C, *et al.*** Chagas disease: an impediment in achieving the millennium development goals in Latin America. *BMC International Health and Human Rights* 2007; **7**: 7.
3. **Schmunis GA.** Epidemiology of Chagas disease in non-endemic countries: the role of international migration. *Memórias do Instituto Oswaldo Cruz* 2007; **102** (Suppl. 1): 75–85.
4. **Rodriguez-Morales AJ, *et al.*** Chagas disease screening among Latin American immigrants in non-endemic settings. *Travel Medicine Infectious Disease* 2008; **6**: 162–163.
5. **ONUSIDA.** 2008 Report on the global AIDS epidemic (http://www.unaids.org/es/KnowledgeCentre/HIVData/GlobalReport/2008/2008_Global_report.asp). Accessed 3 July 2009.
6. **Sartori AM, *et al.*** Exacerbation of HIV viral load simultaneous with asymptomatic reactivation of chronic Chagas' disease. *American Journal of Tropical Medicine and Hygiene* 2002; **67**: 521–523.

7. **Gorlin J, *et al.*** Evaluation of a new *Trypanosoma cruzi* antibody assay for blood donor screening. *Transfusion* 2008; **48**: 531–540.
8. **Flores-Chávez M, *et al.*** Transfusional chagas disease: parasitological and serological monitoring of an infected recipient and blood donor. *Clinical Infectious Diseases* 2008; **46**: 44–47.
9. **Dolcini G, *et al.*** Prevalence of human immunodeficiency virus (HIV)-*Trypanosoma cruzi* co-infection and injectable-drugs abuse in a Buenos Aires health center. *Revista Argentina de Microbiología* 2008; **40**: 164–166.
10. **Eurostat.** Europa 2006. Immigration by sex and citizenship (http://epp.eurostat.ec.europa.eu.). Accessed 28 September 2008.
11. **Spanish Statistical Institute.** Previous dates on immigration 2008 (http://www.ine.es/prensa/np503.pdf). Accessed 28 September 2008.
12. **Caro-Murillo AM, *et al.*** Spanish cohort of naive HIV-infected patients (CoRIS): rationale, organization and initial results [in Spanish]. *Enfermedades Infecciosas y Microbiología Clínica* 2007; **25**: 23–31.
13. **Sartori AM, *et al.*** *Trypanosoma cruzi* parasitemia in chronic Chagas disease: comparison between human immunodeficiency virus (HIV)-positive and HIV-negative patients. *Journal of Infectious Diseases* 2002; **186**: 872–875.
14. **Sartori AM, *et al.*** Manifestations of Chagas disease (American trypanosomiasis) in patients with HIV/AIDS. *Annals of Tropical Medicine and Parasitology* 2007; **101**: 31–50.
15. **Diazgranados CA, *et al.*** Chagasic encephalitis in HIV patients: common presentation of an evolving epidemiological and clinical association. *Lancet Infectious Diseases* 2009; **9**: 324–330.
16. **Cordova E, *et al.*** Reactivation of Chagas disease with central nervous system involvement in HIV-infected patients in Argentina, 1992–2007. *International Journal of Infectious Diseases* 2008; **12**: 587–592.
17. **Corti M.** AIDS and Chagas' disease. *AIDS Patient Care and STDs* 2000; **14**: 581–588.
18. **Corti M, Yampolsky C.** Prolonged survival and immune reconstitution after chagasic meningoencephalitis in a patient with acquired immunodeficiency syndrome. *Revista da Sociedade Brasileira de Medicina Tropical* 2006; **39**: 85–88.
19. **Da-Cruz AM, *et al.*** *Long*-term follow-up of co-infected HIV and *Trypanosoma cruzi* Brazilian patients. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2004; **98**: 728–733.
20. **Gascón J.** Grupo de Trabajo del Taller Enfermedad de Chagas Importada: Un Nuevo Reto de Salud Pública? Diagnosis and treatment of imported Chagas disease. *Medicina Clinica* (*Barcelona*) 2005; **125**: 230–235.
21. **Almeida EA, *et al.*** Chagas' disease and HIV co-infection in patients without effective antiretroviral therapy: prevalence, clinical presentation and natural history. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. Published online: 18 March 2010. doi: 10.1016/j.trstmh.2010.02.004.