Multicenter epidemiological and clinical study on imported Chagas diseases in Alicante, Spain

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Recently, there has been an increase in the number of patients with Chagas disease outside of areas that are generally considered endemic. The aim of this investigation is to describe the clinical profile of a series of patients with Chagas disease in Alicante, Spain, which is a province located on the coast of the Mediterranean Sea. This study was performed at four general hospitals in Alicante between January 2002 and May 2011. A total of 128 patients from seven countries were diagnosed with *Trypanosoma cruzi*. The main country of origin of these patients was Bolivia ($n=101$; 78.9%), and the median of age of these patients was 35 years (range: 0–72 years). Four (3.3%) patients were children under 14 years of age, and 81 (63.3%) were female. Polymerase chain reaction (PCR) was used to analyze 106 patients, 66.0% of whom demonstrated positive PCR results. Visceral involvement was diagnosed in 26.8%: 24.1% demonstrated cardiac involvement, 0.9% demonstrated gastrointestinal involvement, 0.9% demonstrated cardiac and gastrointestinal involvement, and 0.9% demonstrated involvement of the central nervous system. Syncope was found to be associated with cardiomyopathy (28.0% versus 5.2%) (odds ratio: 6.5; 95% confidence interval: 1.5–27.1). Seventy-six patients received treatment with benznidazole, of whom 57 (75.0%) completed the treatment course without significant adverse events and 17.1% discontinued benznidazole due to adverse events. In total, 50% of patients experienced documented adverse reactions. Among patients with positive PCR results before treatment, all demonstrated negative PCR results following treatment. In conclusion, majority of our patients were female Bolivians immigrants, one of four of our patients demonstrated cardiac involvement, and treatment tolerance was poor. It is important to improve the clinical and epidemiological knowledge of Chagas disease in nonendemic with additional multicenter studies in order to determine the magnitude of this problem and provide improved public health and health resource planning.

Keywords: Chagas disease, American trypanosomiasis, *Trypanosoma cruzi* infection, Spain, Immigrant, Bolivia

Introduction

The largest immigration from Latin America to Europe occurred during the last 12 years. In 2011, 12.2% of the total Spanish population was composed of immigrants, and 1.72 million of these immigrants are from Latin America.¹ The most predominant group of Latin American immigrants in Spain is from

Ecuador (23.0%), followed by Colombia (16.9%) and Bolivia (12.3%).¹

Chagas disease, or American trypanosomiasis, is a systemic chronic parasitic infection caused by *Trypanosoma cruzi*. During the last several years, the number of cases has been on the rise in nonendemic countries due to the migration of Latin Americans from endemic areas.^{2,3} In Spain and other European countries, the majority of these studies were mainly performed in referral hospitals or healthcare centers located in cities such as Madrid, Barcelona, or

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Geneva that specialize in tropical medicine and/or international health.^{4–8} The patient often goes to these institutions after a referral from a primary healthcare professional, nongovernmental organization, or relative. The province of Alicante has approximately 1.93 million inhabitants, 4% of whom are originally from Central or South America. In Alicante, the immigrant populations often live in different towns that are separated by more than 30 km. Immigrant patients often go to primary healthcare centers located near their home or place of workplace. Then, if the medical problem is important, they are referred to a hospital near the primary healthcare center. Therefore, in this work, we wanted to describe our experience of treating patients with Chagas disease who were treated at four different hospitals in the province of Alicante using a similar protocol.

Materials and Methods

Study design and setting

This study was a retrospective and analytical epidemiological study and it was performed in four general hospitals in the Alicante province between January 2002 and May 2011. These hospitals consisted of Hospital General Universitario de Alicante, Hospital General Universitario de Elche, Hospital Marina Baixa, and Hospital Clínico Universitario San Juan. All of them are a part of the sanitary public network. None of these centers are referral institutions for the treatment of imported diseases, tropical medicine, and/or international health. During the study period, these hospitals provided medical care to 1.09 million persons from 27 municipalities and of these 71 809 were from Latin America or the Caribbean (6.5%).⁹ To estimate the number of immigrants with *T. cruzi* infection, we used updated country-specific estimates of the prevalence and burden of *T. cruzi* infection that were published by the Pan American Health Organization (PAHO).¹⁰ The application of these prevalence figures makes an implicit assumption that immigrant populations are at the same risk as the overall population that is still living in their country of birth, however this is unlikely to be the case.²

Diagnostic procedures

Two serological tests were performed according to international recommendations.¹¹ The first serological test was enzyme linked immunosorbent assay (ELISA) or immunochromatographic test, different in each microbiological laboratory (Novagnost Chagas IgG; NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany; BioElisa Chagas Biokit, Biokit Barcelona, Spain; Chagas Stat-Pak Assay, Chembio Diagnostic Systems, Medford, USA; Simple CHAGASWB; Operon S.A, Zaragoza, Spain). The second serological test was an indirect immunofluorescent antibody test. It was also prepared in-house, and was done at the

Spanish National Center for Microbiology, Instituto de Salud Carlos III, Madrid, Spain. An individual was considered infected if the results of the two serological tests were positive.¹¹ Polymerase chain reaction (PCR) examinations were performed on samples from seropositive persons (using 121–122 and Tcz1-Tcz2 oligo-nucleotides) at the Spanish National Center for Microbiology, Instituto de Salud Carlos III, Madrid, Spain.¹²

Included variables

The following epidemiological data were recorded: age, sex, country and department of origin, place of residence, rural or urban, in the country of origin; dwelling type (construction materials for floors, walls, etc.) in the county of origin; date of arrival in Spain; any history of blood transfusions, knowledge of Chagas disease, relatives with Chagas disease, and previous diagnosis of Chagas disease in their country or origin. The following clinical symptoms were evaluated for their association with *T. cruzi* infection: chest pain, palpitations, syncope, edema, constipation, gastroesophageal reflux, and dysphagia. These variables were retrospectively collected. The ethics committee of Hospital General Universitario de Elche approved this study.

Others procedures

T. cruzi-infected patients were studied using a protocol that included a 12-lead electrocardiogram (ECG), chest X-ray, and evaluations of hematology, biochemistry levels, and HIV serology. A single reader interpreted the ECG results. Suspicion of cardiac involvement was based on the presence of any of the following ECG criteria and/or echocardiogram criteria: bradycardia (<50 beats/minute); type II, second-degree, or complete atrioventricular blocks; any type of bundle branch block or hemiblock; premature ventricular contractions; or tachyarrhythmia. Echocardiography criteria: left ventricular dysfunction with decreased ejection fraction (<60%); increased left ventricular diastolic diameter; wall movement abnormalities; apical aneurysm; or the presence of thrombotic cardiac cavity with no justifiable cause.^{13,14} Cardiac magnetic resonance imaging was performed according the clinical criteria of the attending physician.

A barium enema and oesophagogram was performed on patients who complained of gastrointestinal symptoms. Disorders of the esophagus were considered if slow transit, retention of the contrast agent, esophageal dilatation, or dolicoesophagus were indicated when evaluating the oesophagogram. The colon was considered abnormal when the sigmoid diameter was >6 cm.¹⁵ Other examinations (holter, cardiac magnetic resonance, etc.) were performed, when necessary, according to the clinical signs and symptoms reported by patients.

Classification of the patients

T. cruzi-infected patients were classified with indeterminate, cardiac, digestive, or mixed clinical forms of Chagas disease. We defined the indeterminate form as a seropositive patient with no evidence of cardiac or gastrointestinal tract involvement. Specific treatment with benznidazole was offered to all infected participants.

Statistical analysis

We analyzed the categorical variables associated with *T. cruzi* infection using the Chi-square or Fisher's exact test; the Wilcoxon rank-sum test was used to compare continuous variables. Odds ratio (OR) with 95% confidence interval (CI) were also calculated. Statistical tests were performed at a two-sided significance level of 0.05. Data obtained from each study participant were entered and analyzed using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA).

Results

A total 128 patients from seven countries were found to be infected with *T. cruzi*. The observed and estimated distribution of *T. cruzi*-infected people by country of origin is shown in Table 1. The main country of origin was Bolivia ($n=101$; 78.9%), followed by Paraguay ($n=11$; 8.6%), Argentina ($n=7$; 5.5%), Colombia ($n=3$; 2.3%), Brazil ($n=2$; 1.6%), and Ecuador ($n=2$; 1.6%). Two cases (1.6%) were congenital cases diagnosed in newborns who were born in Spain to mothers from Bolivia. The number of *T. cruzi* cases diagnosed in Bolivians is half of the estimated number of cases living in the health catchment area of this study according to the estimated prevalence of *T. cruzi* in Bolivia ($n=206$). The number of Latin Americans from other countries diagnosed with *T. cruzi* is hardly lower than the estimated numbers of infected immigrants living in the health catchment area of this study (Table 1).

The median age of the study patients was 35 years (range: 0–72 years). Four patients (3.1%) were children under 14 years of age. Eighty-one (63.3%) were female and 47 (36.7%) were male. Thirty-two patients (25%) were diagnosed during pregnancy in Spain, 25 (19.5%) patients had previous positive serological test results for *T. cruzi*, and 8 (6.3%) were diagnosed during screening in blood bank in Spain. The median number of years spent in residence in Spain was 3.9 years (range: 15 days through 11.1 years). Table 2 shows the basic epidemiological and clinical characteristics of the *T. cruzi*-infected patients. According to the data available (110 patients), 86.0% of patients had knowledge of Chagas disease, 83.5% had lived in a rural area in their country or origin, 69.0% had relatives with Chagas diseases, and 5.7% had received a previous blood transfusion.

Of the 128 *T. cruzi*-infected patients, PCR was performed on 106. Seventy patients (66.0%) had positive PCR results. There were no differences in terms of sex, years spent in residence in Spain, or visceral involvement of Chagas disease. According to the symptoms that are considered suggestive of Chagas disease, 40.0% of patients had palpitations, 26.4% had chest pain, 19.1% were constipated, 15.5% had dyspnoea, 12.2% had gastroesophageal reflux, 10% demonstrated syncope, and 5.5% had dysphagia.

Chest X-rays were performed on 79 patients and indicated cardiomegaly in six patients (7.7%). ECGs were performed on 108 patients, and of these 25 (23.1%) showed various disorders including right bundle branch block (12.0%), supraventricular hemiblock (6.5%), sinus bradycardia (4.6%), complex ventricular extrasystole (3.7%), auriculoventricular block (2.8%), left bundle branch block (1.9%), and sustained ventricular tachycardia (1.9%). Echocardiography was performed on 67 patients, and of these

Table 1 Latin American immigrant with *T. cruzi* infection by country of origin and expected number infected with *T. cruzi* and estimated prevalence of infected immigrant

Country	Number of patients with <i>T. cruzi</i> infection	Number of immigrants of our catchment area*	Prevalence of <i>T. cruzi</i> infection in their country†	Estimated numbers of infected immigrants‡	Estimated prevalence of infected immigrants§
Bolivia	101	3057	6.75	206	3.30
Paraguay	11	3128	2.54	79	0.35
Argentina	7	15 631	4.13	645	0.03
Colombia	3	13 508	0.96	1296	0.02
Ecuador	2	13 663	1.74	2377	0.01
Brazil	2	3058	1.02	31	0.07
Uruguay	0	3866	0.66	25	0.0
Venezuela	0	2828	1.16	33	0.0
Chile	0	2453	0.99	24	0.0
Peru	0	1810	0.69	12	0.0

Note: *Number of immigrant of our catchment area in 2010 according the Instituto Nacional de Estadística.⁹

†Prevalence in their country according the Pan American Health Organization (PAHO) in 2006.¹⁰

‡Estimated numbers of infected immigrants is numbers of immigrants multiplied by prevalence in their country.

§Estimated prevalence of infected immigrants is number of patients with *T. cruzi* infection divided by number of immigrants multiplied by 100.

14.9% demonstrated abnormal findings including 13.3% with wall movement abnormalities, 10.4% with a decreased ejection fraction, and 3% with apical aneurysm. Oesophagogram were abnormal in two of 26 cases (7.7%). The 27 barium enemas that were performed all demonstrated normal findings.

Clinical data are available for 108 patients (20 were lost to follow-up). Visceral involvement was diagnosed in 29 patients (26.8%), 26 (24.1%) had cardiac involvement, 1 (0.9%) had gastrointestinal involvement, 1 (0.9%) had cardiac and gastrointestinal involvement, and 1 (0.9%) had central nervous system involvement. Of 27 patients with cardiac disorder, five patients had pacemakers, one patient had received a heart transplant, and one patient was waiting for a heart transplant. There were no significant differences among patients with cardiac involvement in terms of sex; however, the patients with cardiac involvement were discretely older (median: 39 years; range: 21–52 years) than those without cardiomyopathy (median: 33.4 years; range: 0–72 years; $P=0.09$). The patients with cardiac involvement, as determined by ECG and/or echocardiogram, more frequently experienced syncope and dyspnea than those without cardiac involvement, as shown by the results of the univariate analysis (Table 3). Multivariate logistic regression analysis showed that only syncope was associated with cardiomyopathy (OR: 6.5; 95% CI: 1.5–27.1).

Thirty-two women were pregnant (25%), and there were two cases (6.2%) of vertical transmission that were determined to have occurred in Spain. The newborns were asymptomatic at the birth. One patient was male HIV-infected and had a reactivation of Chagas disease with subacute meningitis. In addition, 30.2% of patients had eosinophilia. From 39 cases of eosinophilia, strongiloidiasis was diagnosed in 12 cases, but all of cases were treated with albendazole or ivermectin.

Table 2 Epidemiological characteristics and symptoms of *T. cruzi*-infected people

Characteristics	Number/total number of cases (%)
Living in rural area	66/79 (83.5)
Relatives with Chagas disease	49/71 (69.0)
Knowledge of Chagas disease	74/86 (86.0)
Previous blood transfusion	6/107 (5.6)
Previous <i>T. cruzi</i> serology positive	39/114 (34.2)
Diagnosis during pregnancy screening	32/128 (25.0)
Clinical symptoms	
Asymptomatic	50/110 (45.5)
Palpitation	44/110 (40.0)
Chest pain	29/110 (26.4)
Constipation	21/110 (19.1)
Dyspnoea	17/110 (15.5)
Gastroesophageal reflux	11/90 (12.2)
Syncope	11/110 (10.0)
Dysphagia	6/110 (5.5)
Edemas	3/110 (2.7)

Out of the 108 patients for whom clinical data were available, 76 patients received treatment with 5 mg/kg/day benznidazole (100 mg/tablet, Radanil®; Roche) during 45–60 days. Fifty-seven (75.0%) patients completed the treatment course without significant adverse events or with only mild adverse events that did not lead to the discontinuation of treatment. Thirteen (17.1%) discontinued benznidazole due to adverse events and six discontinued treatment due to other factors (five patients were lost on follow-up after starting treatment and one was lost due to acute cholecystitis).

A safety and tolerability analysis was performed on 70 of 76 patients (those patients who had completed treatment or had already discontinued treatment due to adverse reactions). In total, 35 of 70 (50%) patients had documented adverse reactions and 13 (18.6%) stopped treatment because of adverse side effects. Age and sex were not significantly associated with the occurrence of adverse reactions or the interruption of treatment. Of the 35 patients who developed toxicity, the most common side effects included cutaneous toxicity (e.g., morbilliform rash, pruritus, occasional mucosal involvement) in 74.5%, gastrointestinal symptoms (e.g., nausea, vomiting, gastric pain, anorexia) in 11.4%, and nervous system disturbances (e.g., dysesthesia or paresthesia) in 8.6%, and increased hepatic enzyme levels in 5.7%. Among the 50 patients with positive PCR results before treatment, all demonstrated negative PCR results following treatment.

Discussion

Recently, there has been an increase in the number of patients with Chagas disease outside of areas generally considered endemic for this disease, particularly in Spain where, until recently, the disease was hardly ever diagnosed.^{2,5,7,8} Most of the patients included in this study were from Bolivia (currently the country with the highest prevalence of Chagas disease),¹⁰ which is

Table 3 Univariate analysis of variables associated with cardiomyopathy in 106 *T. cruzi* patients

	Cardiomyopathy N (%)	Non- cardiomyopathy N (%)	P value
Sex			0.622
Male	11 (40.7)	28 (35.4)	
Female	16 (59.3)	51 (64.6)	
Age			0.091
Median (range)	39 (21–52)	33.4 (0–72)	
Palpitation			0.107
No	11 (44.0)	48 (62.3)	
Yes	14 (56.0)	29 (37.7)	
Chest pain			0.140
No	15 (60)	58 (75.3)	
Yes	10 (40)	19 (24.7)	
Syncope			0.001
No	18 (72.0)	73 (94.8)	
Yes	7 (28.0)	4 (5.2)	
Dyspnea			0.029
No	17 (68.0)	68 (88.3)	
Yes	8 (32.0)	9 (11.7)	

similar to the findings of other studies performed in Spain.^{2,5,7,8,16,17} The second most common country of origin was Paraguay, followed by Argentina. According to PAHO,¹⁰ the seroprevalence of *T. cruzi* infection in the global Paraguayan population is 2.54%, which is less than among Argentines. Although there are less Paraguayans living in our area, the number of cases of *T. cruzi* infection is higher among these individuals than among Argentines. In this study, the number of *T. cruzi*-infected Bolivians is more than others nationalities to the number of cases of *T. cruzi* estimated in catchment area. The number of *T. cruzi* Paraguayans and Argentines is less than the number of cases of *T. cruzi* estimated in our catchment area. Therefore, it should be necessary a implementing diagnostic strategies, and health resource planning in Latin American population.¹⁸

The rate of cardiac involvement (25.4%) was slightly higher than that reported in the Spanish literature (18.6–19%).^{5,8} The diagnosis of Chagas cardiomyopathy is still based on the simple and nonspecific criteria of an increased cardiothoracic ratio (>0.5), abnormal ECG, or abnormal echocardiogram.^{13,14} These parameters are far from perfect and may misclassify patients, thereby resulting in mismanagement. New initial screening parameters and the measurement of brain natriuretic peptide in combination with a comprehensive analysis of diastolic function could allow the earlier detection of cardiac involvement in patients with Chagas disease.¹⁹ The symptoms and signs of Chagas cardiomyopathy that are typically seen on physical examination are the same as for cardiomyopathy in general, but none are characteristic of Chagas disease.¹³ Moreover, in this series the patients with cardiac involvement experienced syncope more often than non-cardiac involvement after adjusting for other factors. For that, syncope is a clinical criterion for suspicion of cardiac involvement.

Gastrointestinal abnormalities were not as common as cardiomyopathy. In our study, the rate of gastrointestinal involvement (1.9%) was slightly lower than that reported in the Spanish literature (5–9%).^{5,8} This can be explained by the fact that in our study the oesophagogram and barium enema were only performed on patients with gastrointestinal symptoms. Based on esophageal manometry, the prevalence of esophageal involvement (incomplete relaxation of the lower esophageal sphincter) is 11%.²⁰ Using new procedures to diagnose cardiomyopathy and gastrointestinal disorders in association with Chagas diseases will allow the diagnosis of more cases with visceral involvement.

The effectiveness of benznidazole for eradicating *T. cruzi* during the chronic phase of the disease is controversial.^{21,22} Nevertheless, studies in Argentina have reported that treatment with benznidazole can

reduce the progression of Chagas disease and increase the odds of long-term negative seroconversion in patients with chronic disease but without heart failure.²³ In our study, as in other studies,⁸ all of the patients with positive PCR results before treatment for *T. cruzi* demonstrated negative results following treatment. Nevertheless, Pinazo *et al.*²⁴ and Murcia *et al.*⁷ found that 19 and 6.9% of patients, respectively, who were treated with benznidazole remained positive after completing a full treatment regimen. It is important to remember that a negative PCR result does not guarantee a parasitological cure, because parasitemia may fluctuate during the chronic phase of infection.^{7,25} In this study, follow-up treatment was not evaluated.

Benznidazole can have harmful side effects, which in some cases is severe enough for patients to discontinue treatment. In this study, tolerance was generally poor with a frequency of adverse reactions similar that described in other studies (50%).^{8,24,26} The proportion of adverse effects due to cutaneous involvement was 75%. In other Spanish studies, the rate of adverse side effects ranges from 68.7–88%.^{8,24,26} These rates are even higher than those previously reported in Latin American countries (20–25%).²⁷ The rate of discontinuation in this study due to side effects was 18.6%, and in other Spanish studies the rate has been reported as 5.6–29.7%.^{8,24,26} We found no association between sex, age, and the appearance of adverse effects, similar to the series by Pérez-Ayala *et al.*⁸ and Carrilero *et al.*²⁶

T. cruzi infection is no longer limited to the Latin American continent and has emerged in Western Europe.² The majority of studies on Chagas disease were performed at referral healthcare centers or hospitals located in large cities. The conclusions and lessons that can be drawn from our study, which was performed at four healthcare centers, are similar to those that can be drawn from studies performed at larger centers. The majority of our patients were female Bolivians immigrants, high percentage of the Paraguayan population affected, one of four of our patients demonstrated cardiac involvement, and treatment tolerance was poor. Additional multi-center studies with consensus protocol to compare data between different centers are necessary in order to determine the magnitude of this problem and provide improved public health and health resource planning. Finally, challenges of Chagas disease in Spain continue.

References

- 1 Instituto Nacional de Estadística. Avance del Padrón Municipal a 1 de enero 2011. Madrid: INE; 2011 [Internet]. [cited 2011 Dec 12]. Available from: <http://www.ine.es/prensa/np648.pdf>
- 2 Gascon J, Bern C, Pinazo MJ. Chagas disease in Spain, the United States and other non-endemic countries. *Acta Trop*. 2010;115:22–7.

- 3 Schmunis GA, Yadon ZE. Chagas disease, a Latin American health problem becoming a world health problem. *Acta Trop*. 2010;115:14–21.
- 4 Jackson Y, Angheben A, Carrilero Fernandez B, Jansa i Lopez del Vallado JM, Jannin JG, Albajar-Viñas P. Management of Chagas disease in Europe. Experiences and challenges in Spain, Switzerland and Italy. *Bull Soc Pathol Exot*. 2009;102:326–9.
- 5 Muñoz J, Gómez i Prat J, Gállego M, Gimeno F, Treviño B, López-Chejade P, et al. Clinical profile of *Trypanosoma cruzi* infection in a non-endemic setting: immigration and Chagas disease in Barcelona (Spain). *Acta Trop*. 2009;111:51–5.
- 6 Jackson Y, Alirol E, Getaz L, Wolff H, Combescure C, Chappuis F. Tolerance and safety of nifurtimox in patients with chronic chagas disease. *Clin Infect Dis*. 2010;5:e69–75.
- 7 Murcia L, Carrilero B, Muñoz MJ, Iborra MA, Segovia M. Usefulness of PCR for monitoring benznidazole response in patients with chronic Chagas' disease: a prospective study in a non-disease-endemic country. *J Antimicrob Chemother*. 2010;65:1759–64.
- 8 Pérez-Ayala A, Pérez-Molina JA, Norman F, Navarro M, Monge-Maillo B, Díaz-Menéndez M, et al. Chagas disease in Latin American migrants: a Spanish challenge. *Clin Microbiol Infect*. 2011;17:1108–13.
- 9 Instituto Nacional de Estadística. Revisión del padrón municipal 2010. Datos por municipios. 03-Alicante/Alacant. Población nacida en el extranjero por sexo, municipios y lugar de nacimiento. Madrid: INE; 2010 [cited 2011 Dec 29]. Available from: <http://www.ine.es/jaxi/tabla.do?path=/t20/e245/p05/a2010/10/&file=00003004.px&type=pcaxis&L=0>
- 10 Organización Panamericana de la Salud. Estimación cuantitativa de la enfermedad de Chagas en las Américas. Montevideo, Uruguay, Organización Panamericana de la Salud. Washington DC: PAHO Publishing; 2006. 1–28 [OPS/HDM/CD/425-06].
- 11 WHO — World Health Organization. Control of Chagas Disease, WHO Technical Report Series 811. Geneva: WHO; 2002. [cited 2010 May 01]. Available from: http://whqlibdoc.who.int/trs/WHO_TRS_905.pdf
- 12 Flores-Chávez M, Fernández B, Puente S, Torres P, Rodríguez M, Monedero C, et al. Transfusional chagas disease: parasitological and serological monitoring of an infected recipient and blood donor. *Clin Infect Dis*. 2008;46:e44–7.
- 13 Gascón J, Albajar P, Cañas E, Flores M, Gómez i Prat J, Herrera RN, et al. Diagnosis, management and treatment of chronic Chagas' heart disease in areas where *Trypanosoma cruzi* infection is not endemic. *Rev Esp Cardiol*. 2007;60:285–93.
- 14 Sosa-Estani S, Viotti R, Segura EL. Therapy, diagnosis and prognosis of chronic Chagas disease: insight gained in Argentina. *Mem Inst Oswaldo Cruz*. 2009;104 Suppl 1:167–80.
- 15 de Oliveira RB, Troncon LE, Dantas R, Menghelli UG. Gastrointestinal manifestations of Chagas' disease. *Am J Gastroenterol*. 1998;93:884–9.
- 16 Muñoz J, Coll O, Juncosa T, Vergés M, Vergés M, del Pino M, Fumado V, et al. Prevalence and vertical transmission of *Trypanosoma cruzi* infection among pregnant Latin American women attending 2 maternity clinics in Barcelona, Spain. *Clin Infect Dis*. 2009;48:1736–40.
- 17 Ramos JM, Milla A, Sánchez V, Vergés M, Toro C, Gutiérrez F. Prenatal screening for *Trypanosoma cruzi* and human T lymphotropic virus types 1 and 2 in pregnant Latin American women. *Enferm Infecc Microbiol Clin*. 1999;27:165–7.
- 18 Ramos JM, Ponce Y, Gallegos I, Flores-Chávez M, Cañavate C, Gutiérrez F. *Trypanosoma cruzi* infection in Elche (Spain): comparison of the seroprevalence in immigrants from Paraguay and Bolivia. *Pathog Glob Health*. 2012;106:102–6.
- 19 Garcia-Alvarez A, Sitges M, Pinazo MJ, Regueiro-Cueva A, Posada E, Poyatos S, et al. Chagas cardiomyopathy: the potential of diastolic dysfunction and brain natriuretic peptide in the early identification of cardiac damage. *PLoS Negl Trop Dis*. 2010;21:4 pii:e826.
- 20 Pérez-Ayala A, Pérez-Molina JA, Norman F, Monge-Maillo B, Faro MV, López-Vélez R. Gastro-intestinal Chagas disease in migrants to Spain: prevalence and methods for early diagnosis. *Ann Trop Med Parasitol*. 2011;105:25–9.
- 21 Marin-Neto JA, Rassi A Jr, Morillo CA, Avezum A, Connolly SJ, Sosa-Estani S, et al. BENEFIT Investigators. Rationale and design of a randomized placebo-controlled trial assessing the effects of etiologic treatment in Chagas' cardiomyopathy: the BENznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT). *Am Heart J*. 2008;156:37–43.
- 22 Pérez-Molina JA, Pérez-Ayala A, Moreno S, Fernández-González MC, Zamora J, López-Vélez R. Use of benznidazole to treat chronic Chagas' disease: a systematic review with a meta-analysis. *J Antimicrob Chemother*. 2009;64:1139–47.
- 23 Viotti R, Vigliano C, Lococo B, Bertocchi G, Petti M, Alvarez MG, et al. Long-term cardiac outcomes of treating chronic Chagas disease with benznidazole versus no treatment: a nonrandomized trial. *Ann Intern Med*. 2006;144:724–34.
- 24 Pinazo MJ, Muñoz J, Posada E, López-Chejade P, Gállego M, Ayala E, et al. Tolerance of benznidazole in treatment of Chagas' disease in adults. *Antimicrob Agents Chemother*. 2010;54:4896–9.
- 25 Gomes YM, Lorena VM, Luquetti AO. Diagnosis of Chagas disease: what has been achieved? What remains to be done with regard to diagnosis and follow up studies? *Mem Inst Oswaldo Cruz*. 2009;104 (Suppl 1):115–21.
- 26 Carrilero B, Murcia L, Martínez-Lage L, Segovia M. Side effects of benznidazole treatment in a cohort of patients with Chagas disease in non-endemic country. *Rev Esp Quimioter*. 2011;24:123–6.
- 27 Viotti R, Vigliano C, Lococo B, Alvarez MG, Petti M, Bertocchi G, et al. Side effects of benznidazole as treatment in chronic Chagas disease: fears and realities. *Expert Rev Anti Infect Ther*. 2009;7:157–63.