Clinical Microbiology and Infection 22 (2016) 788–792

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Serodiscordance in chronic Chagas disease diagnosis: a real problem in non-endemic countries

Z. Moure [1,*], A. Angheben [2,3], I. Molina [4], F. Gobbi [2,3], M. Espasa [1], M. Anselmi [5], F. Salvador [4], S. Tais [6], A. Sánchez-Montalvá [4], T. Pumarola [1], P. Albajar-Viñas [7], E. Sulleiro [1]

[1] Microbiology Department, Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, PROSICS, Barcelona, Spain
[2] Centre for Tropical Diseases, Hospital 'Sacro Cuore–Don Calabria', Negrar, Italy
[3] Global Health Centre of Tuscany Region, Florence, Italy
[4] Infectious Diseases Department, Vall d'Hebron University Hospital, PROSICS, Barcelona, Spain
[5] Centro de Epidemiologia Comunitaria y Medicina Tropical, Esmeraldas, Ecuador
[6] Service of Epidemiology and Laboratory for Tropical Diseases, Hospital 'Sacro Cuore—Don Calabria', Negrar, Italy
[7] Department of HIV/AIDS, Tuberculosis, Malaria and Neglected Diseases, Control of Neglected Tropical Diseases Department, WHO, Geneva, Switzerland

ARTICLE INFO

Article history:
Received 25 February 2016
Received in revised form
2 June 2016
Accepted 6 June 2016
Available online 16 June 2016

Editor: E. Bottieau

Keywords:
Chagas
Chronic
Inconclusive
Non-endemic
Serology

ABSTRACT

According to the WHO, chronic Chagas disease (CD) diagnosis is based on two serological techniques. To establish a definitive diagnosis, the results must be concordant. In cases of discordances, the WHO proposes repeating serology in a new sample, and if results remain inconclusive, a confirmatory test should be performed. This study, conducted at two Tropical Medicine Units in Europe over 4 years, aims to assess the diagnostic yield of TESA- (trypomastigote excreted–secreted antigens) blot as a confirmatory technique in patients with inconclusive and discordant results. Of 4939 individuals screened, 1124 (22.7%) obtained positive results and 165 (3.3%) discordant results. Serology was repeated in 88/165 sera and discrepancies were solved in 25/88 (28.4%) cases. Patients without a definitive diagnosis were classified in two different groups: Group 1, including patients with inconclusive results despite retesting ($n = 63$), and Group 2, including patients with discordant results not retested ($n = 77$). TESA-blot was performed for all of Group 1 and 39/77 of Group 2 and was positive for 33/63 (52.4%) and 21/39 (53.8%), respectively. Analysis of Group 1 results showed a moderate agreement between results of the ELISA based on native antigen and TESA-blot (κ 0.53). In contrast, a clear disagreement was observed between the ELISA based on recombinant antigens and TESA-blot (κ <0). A sizeable proportion of patients are suspected to have CD with inconclusive results or in whom re-testing is not feasible. TESA-blot was positive in half of these patients, highlighting the need for a confirmatory assay in European centres caring for exposed individuals. **Z. Moure, CMI 2016;22:788**

Introduction

Chagas disease (CD) is a zoonotic infection caused by the flagellate protozoan *Trypanosoma cruzi*. CD is endemic in continental Latin America, where an estimated 6–7 million people are infected, and nearly 60 million people are at risk of infection [1,2]. Moreover, in recent decades CD has become a global issue as a consequence of increased migration from endemic to non-endemic areas [3,4]. Spain, followed by Italy, receives the most Latin American immigrants in Europe [5,6].

CD diagnostics depend on the phase of infection. In acute phase, the best diagnostic strategy relies on direct parasitological techniques and, recently, on PCR. The chronic indeterminate phase is characterized by low or undetectable parasitaemia and parasitological examinations, even PCR, are often useless. According to the WHO, chronic CD diagnosis is based on detection of anti-*T. cruzi* antibodies by two different serological techniques performed in parallel, unless a highly sensitive and specific technique becomes available for use alone [7]. Indirect immunofluorescence, ELISA, or haemagglutination assay are usually recommended [7–9].

* Corresponding author. Z. Moure, Vall d'Hebron Universitary Hospital, Microbiology Department, P. de la Vall d'Hebron 119-129, 08035 Barcelona, Spain.
E-mail address: zmoure@vhebron.net (Z. Moure).

http://dx.doi.org/10.1016/j.cmi.2016.06.001
1198-743X/

To establish a definitive diagnosis, the results of both techniques must be concordant. In cases of discordance, the WHO proposes repeating serology using a new serum sample, and if results remain inconclusive a confirmatory test such as PCR or Western blotting should be performed. However, CD PCR is highly variable in its limit of detection, which depends on the presence of the parasite in blood and is currently not considered efficient for diagnosing chronic CD [7,10,11]. The commercial Western blot, TESA-blot (Biomérieux, RJ, Brasil), is an immunoblotting assay that uses secreted and excreted trypomastigote antigens. TESA-blot has sensitivity and specificity values close to 100% and is considered an excellent serological confirmatory test [12,13].

We present a study on discordant cases of CD seen during a 4-year period at two reference Tropical Medicine Units in Europe. This study aims to assess the diagnostic yield of TESA-blot as a confirmatory technique in patients with inconclusive and discordant results.

Materials and methods

This is a retrospective observational study performed at Vall d'Hebron University Hospital (HUVH), Barcelona, Spain, and Centre for Tropical Diseases, Sacro Cuore Hospital (CTD), Negrar, Italy from January 2010 to December 2014. CD disease screening was performed in all adult non-Caribbean Latin-American individuals who attended at the two centres during this period and accepted.

To analyse patients without a definitive diagnosis, the inclusion criteria were: adults >18 years old with at least one discordant *T. cruzi* serology, no co-morbidities conditioning immunosuppression, and no previous specific trypanocidal treatment. Clinical and epidemiological data were collected when available: age, gender, country of origin and visceral involvement. Cardiac involvement was assessed by 12-lead electrocardiogram and chest X-ray, and gastrointestinal involvement was assessed through oesophagogram and barium enema. The study protocol was approved by the ethical review boards of both hospitals.

Serological diagnosis

Serum samples were tested by two ELISAs simultaneously, one based on a recombinant antigen (r-ELISA), and the other on a native antigen (n-ELISA). The commercial kit Bioelisa Chagas (Biokit, Lliça d'Amunt, Spain) for r-ELISA was used in both laboratories. In relation to the native antigen, EIA ORTHO *T. cruzi* ELISA Test System (Johnson and Johnson, New Brunswick, NJ, USA) was carried out at the HUVH, and ELISA Chagas III Test (BiosChile, Santiago, Chile) was performed at the CTD. All screening tests used in the study have a reported sensitivity and specificity close to 100% [14,15].

Results were expressed as the index between the absorbance of the test serum and the threshold value. Tests were considered negative if the index was <0.9, equivocal if ≥0.9 and < 1, and positive if ≥1. In case of discordant results, serology was repeated using a new sample at 4–6 months whenever possible. If results remained discordant, they were finally considered inconclusive.

RT-PCR

When possible, *T. cruzi* real-time PCR was performed in guanidine hydrochloride pre-treated blood samples of patients with inconclusive results as described elsewhere [16]. All of the samples were analysed in duplicate and considered valid when the internal control was amplified. Results were positive when at least one of the two cycle thresholds (Ct) for the *T. cruzi* target was <40.

Immunoblotting

The commercial Western blot test, TESA-blot, with reported sensitivity and specificity values close to 100%, was performed for all inconclusive results, and the best-studied discordant sera as previously described [12,17]. The presence of bands in the 120- to 200-kDa molecular mass region indicated a positive result and the absence of such bands indicated a negative result.

Due to the cross-reactivity of chagasic sera with other organisms, mainly with *Leishmania* spp., three serum samples from patients infected with the latter organism were tested by TESA-blot. Three sera from confirmed chagasic patients were used as positive controls and three parasite-negative sera were used as negative controls.

Statistical analysis

SPSS v17 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Categorical data are presented as absolute numbers and proportions, and continuous variables are expressed in terms of means and ranges or 95% CI. Chi-square test, with Fisher correction if necessary, was used for discrete variables and the Student's *t* test was used for continuous variables when required. The correlation between results of TESA-blot with those of r-ELISA and with n-ELISA was assessed using a κ coefficient.

Results

During the study period, 4939 Latin American immigrants were screened for CD in both centres (2629 from CTD and 2310 patients from HUVH). Initially, 22.7% (1124/4939) of patients were confirmed as being infected with *T. cruzi* as both tests performed at each hospital were positive. A total of 73.9% (3650/4939) of patients had negative diagnoses, and 3.3% (165/4939) had initially discordant results. It was possible to repeat serology in 88 of this subgroup of patients and discrepancies were resolved in 25/88 (28.4%) cases (seven were confirmed positive and 18 negative). Finally, we classified patients without a definitive screening diagnosis for CD into two groups:

- Group 1: patients with inconclusive results despite repeating serology using a new sample ($n = 63$).
- Group 2: patients with discordant results in whom it was not possible to repeat serology using a new sample ($n = 77$).

Epidemiological, clinical, and analytical data of Group 1 and Group 2 are summarized in Table 1.

Information about the index values of the ELISAs was available only for the Spanish cohort of infected patients ($n = 616$; r-ELISA index mean 4.78 ± 2.21, n-ELISA index mean 4.76 ± 1.65). When comparing ELISA indices of Group 1 and Group 2, each, with ELISA indices of the infected cohort, significant differences were found for both groups ($p < 0.0001$).

All sera from patients with inconclusive results (Group 1) were tested by TESA-blot. The test was positive in 33 (52.4%) and negative in 30 (47.6%) of the 63 samples.

Regarding patients with discordant results (Group 2), TESA-blot was performed in 39/77 individuals. The test was positive in 21/39 (53.8%) and negative in 18/39 (46.2%) of the samples.

Following the WHO algorithm, our results are presented in Fig. 1.

When we analysed TESA-blot-positive and -negative groups in Group 1, patients of Bolivian origin were significantly higher in the first group (p 0.003); however, no significant differences in relation to age or gender were found between the two groups.