High level of coinfection HIV-*Tripanosoma cruzi* in vulnerable population in Buenos Aires, Argentina

A. Benchetrit[1], G. Andreani[2], M.M. Avila[2], D. Rossi[3], J. Benetucci[1], R. Casco[4], M. Losso[5], P. Cahn[6], A.M. De Rissio[7], M. Weissenbacher[8], L. Martinez Peralta[8] & G. Dolcini[2].

Hospital de Enfermedades Infecciosas "Dr. Francisco J. Muñiz"[1], Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS, UBA CONICET)[2], Intercambios Asociación Civil[3], Hospital de Clinicas"Jose de San Martın"[4], Hospital Ramos Mejía[5], Hospital Juan Fernandez[6], Instituto Fatala Chaben[7], Instituto de Microbiología y Parasitología Médica, Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Tecnológicas (IMPaM, UBA CONICET)[8]

ABSTRACT

The coinfection with *Tripanosoma cruzi* (*T. cruzi*) and HIV is a relevant clinical event. The goal of this study was to establish the frequency of coinfection in at-risk populations for HIV infection from Argentina. A total of 280 HIV positive serum samples from HIV seroprevalence studies in HIV at-risk groups were tested for *T.cruzi* infection. Of those samples, 8 were positive for *T. cruzi* (coinfection rate: 2.9%); 6 of them came from HIV positive intravenous drug users (IDUs)(rate: 7.8%). In view of these results, all HIV infected patients in Argentina should undergo *T. cruzi* diagnosis, especially IDUs.

INTRODUCTION

Chagas disease stands as a relevant chronic infectious process from the public health point of view. It is estimated that 6-7 million people areinfected, mostly in Latin America; generating substantial costs[1].The number of infected persons in Argentina is 2.3 million people[2]. Although historically a rural disease, a large amount of people infected with *Tripanosoma cruzi* (*T. cruzi*) in Argentina has migrated to urban centers in the search of a better position, promoting a coexistence with the mainly urban HIV epidemic in Argentina. This has led to greater number of HIV – *T. cruzi* coinfected patients[3].

Not only do both infections share routes of transmission, but they also have important effects on their evolution. It has been described that fluctuations on *T. cruzi* parasitemia affects HIV viral loads[4]. Furthermore, HIV patients with profound immunosuppression may reactivate Chagas disease with encephalitis and/or myocarditis[5].

The HIV – *T. cruzi* coinfection prevalence in endemic countries rates from 1.22 to 7.1%[6-9]. In non-endemic countries, Spain has a coinfection prevalence of 1.9 to 3.9% in larger studies[10-11], representing mainly immigrants from endemic countries with chronic Chagas disease. In Argentina the largest studies found a coinfection prevalence of 1.22 to 4.2%, being higher in intravenous drug users: 3.7 to 8.9%[6, 8].The aim of this study was to detect HIV- *T. cruzi* coinfection frequency in at-risk HIV infection populations from Argentina.

MATERIALS AND METHODS

Serum samples from 4 HIV seroprevalence cross sectional studies conducted in Buenos Aires city between 2000 and 2004 in different HIV at-risk groups were tested for *T. cruzi* infection. Studied groups were 174 intravenous drug users (IDUs), 504 non-injecting cocaine users (NICUs), 801 patients from sexually transmitted infections (STI) clinics, and 694 men who have sex with men (MSM). From those studies, a group of HIV infected samples were randomly selected in order to be tested for *T. cruzi*. Samples were also tested for hepatitis B (HBV), hepatitis C (HCV) and human T-lymphotropic viruses (HTLVs).

Participants were enrolled in the study if they were 18 years of age or older, from Buenos Aires City or surroundings and able and willing to provide informed consent, answer the study questionnaire, and be tested for blood borne pathogens, such as HIV, *T. cruzi*, HBV, HCV, and HTLV-I and -II antibodies. Questionnaires focused on gathering information on sociodemographic aspects, sexual practices, drug use, and relationship with the health system.

Pretest and posttest counseling for HIV, *T. cruzi*, HCV, HBV, and HTLV-I and HTLVII were offered to all participants. Participants with positive serological tests were referred to an infectious diseases department for appropriate clinical treatment and monitoring. Free HBV immunization for those persons with no HBV antibodies was offered. International and national ethics guidelines for biomedical research involving human

subjects were strictly followed[12]. The studies were approved by the ethics committees from each institution.

Plasma samples were analyzed by means of ELISA and agglutination techniques for detection of HIV (Genscreen HIV1/2 version 2, Bio-Rad; SFD HIV 1/2 PA, Bio-Rad, Fujirebio); HBV (Core CM AxSym System; surface antigen to HBV[HBsAg], version 2, AxSym System; Abbott); HCV (HCV version 3.0, AxSym System; Abbott); HTLV-I and HTLV-II (Platelia HTLV-I New, Bio-Rad; Serodia HTLV-I, Fujirebio). *T. cruzi* infection was detected with ELISA, IHA, and IFA, using "in-house" antigens, compliant with domestic and international rules. The parasite antigens used in those methods vary widely, from whole cells for IFA to crude cell extracts for IHA and ELISA. The sensitivity of the tests varies between 99.0% and 99.8%. When two or three tests were performed simultaneously, the sensitivity ranges from 99.7% to 100% and the specificity from 97.4% to 97.9%. Titers equal or higher than 0.200 of optical density at 490 nm for ELISA, and 1/32 for IHA and IFA were considered reactive for *T. cruzi* infection [13]. Subjects positive on at least two of these tests were considered to be infected.

Differences among rates of prevalence were analyzed by Fisher's exact test. Data were entered and analyzed with Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. InfoStatversión 2015. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar

Comentario [U1]: Poner en bibliografía?

RESULTS

A total of 280 HIV positive serum samples were studied. These samples came from 77 IDUs, 28 NICUs, 51 STI patients and 124 MSM. Of the 280 samples tested 8 were positive for *T. cruzi*, giving a coinfection frequency of 2.9%. Of these 6 came from HIV positive IDUs, and 2 from HIV positive STI samples, giving a coinfection rate of 7.8% and 3.9% in each group (Table). No coinfection was detected in NICU or MSM (Table 1). *T. cruzi* infection was compared between HIV positive IDUs (n = 77) and HIV positive non-IDUs (n = 208) being significantly more frequent in HIV positive IDUs (7.8% vs. 1%, $p<0.05$), as shown in the figure.

All six IDUs with positive *T. cruzi* serology were men, with a mean age of 30 years (range 27-33 years). The socioeconomic level was low, none of them finished high school and all were unemployed. The mean age of first intravenous drug use was 17 years (range 14-27 years), the most usual drug used was cocaine (6/6), and only one subject answered not sharing needles at the moment of the questionnaire. All six of them also had positive HCV serology, and five had positive anti HBV core serology, only one of them had active HBV. HTLV-II coinfection was seen in 4 of the 6 HIV-*T. cruzi* coinfected IDUs.

DISCUSSION

Chagas disease vector control programs have limited this way of transmission in many countries of Latin America[13]. In the same way blood testing implementation for *T. cruzi* infection has diminished blood related infections[14]. These control measures have led to a relative increase in other ways of transmission, such as congenital transmission[15]. Needle sharing in IDUs has been described as a potential *T. cruzi* way of transmission in urban environments, and could have a significant role in Chagas disease spread, especially in non-endemic areas[6, 8, 16].

The overall Chagas disease frequency found in our study (2.9%) was coincident with the prevalence found in blood donors in Buenos Aires City (2.7%) [14]. In a similar way to previous reports, we found a significantly higher rate of *T. cruzi* infection in IDUs[6, 8], highlighting the relationship between intravenous drug use and Chagas transmission in HIV infected patients, as with other blood related infectious diseases such as HCV, HBV and HTLV-II. Given the retrospective nature of our study some epidemiological data could not be assessed. Although the current residence of all subjects could be assessed, no information on their provenance, travel or blood transfusion history was given. Neither could data from their Chagas disease involvement or reactivation be found.

In view of the submitted results, we believe all HIV infected patients in the region should undergo *T. cruzi* infection studies, regardless if they come from endemic areas or not, especially in IDUs. More studies regarding this coinfection and the relevance of intravenous drug use should be performed.

REFERENCES

1- World Health Organization (WHO). Chagas disease (American trypanosomiasis) (Cited 2015 August 20). Available at: http://www.who.int/mediacentre/factsheets/fs340/en/

2- Esquivel ML, Segura EL. Calculating the number of patients with Chagas disease in Argentina. Medicina (B Aires). 1994;54(1):91-2.

3- Vaidian AK, Weiss LM, Tanowitz HB. Chagas' disease and AIDS. KinetoplastidBiol Dis. 2004;3(1):2.

4- Sartori AM, Caiaffa-Filho HH, Bezerra RC, et al. Exacerbation of HIV viral load simultaneous with asymptomaticreactivation of chronic Chagas' disease. Am J Trop Med Hyg. 2002;67(5):521-3.

5- Sartori AM, Ibrahim KY, NunesWestphalen EV, et al. Manifestations of Chagas disease (American trypanosomiasis) in patients with HIV/AIDS. Ann Trop Med Parasitol. 2007;101(1):31-50.

6- Scapellato PG, Bottaro EG, ScapellatoJl, et al. ¿Es posible la transmision de la enfermedad de Chagas mediante el hábito de compartir jeringas entre pacientes HIV+ adictos a drogas?. Medicina (B. Aires). 2006; 66(6):595-6.

7- Almeida EA, Lima JN, Lages-Silva E, et al. Chagas' disease and HIV co-infection in patients without effective antiretroviral therapy: prevalence, clinical presentation and natural history.Trans R Soc Trop Med Hyg. 2010;104(7):447-52.

8- Dolcini G, Ambrosioni J, Andreani G, et al. Prevalence of human immunodeficiency virus (HIV)-*Trypanosomacruzi* co-infection and injectable-drugs abuse in a Buenos Aires health center. RevArgent Microbiol. 2008;40(3):164-6.

9- Diez MS, Nocito I, de Frade AR, et al. Serological evidence of cytomegalovirus, hepatitis B and C, Epstein-Barr virus, *Toxoplasma gondii*, *Trypanosomacruzi* and *Treponemapallidum* in HIV infected patients. Medicina (B Aires). 2001;61(3):378-80.

10- Llenas-García J, Hernando A, Fiorante S, et al. Chagas disease screening among HIV-positive Latin American immigrants: an emerging problem. Eur J ClinMicrobiol Infect Dis. 2012;31(8):1991-7.

11- Salvador F, Molina I, Sulleiro E, et al. Tropical diseases screening in immigrant patients with human immunodeficiency virusinfection in Spain. Am J Trop Med Hyg. 2013;88(6):1196-202.

12- International Ethical Guidelines for Biomedical Research Involving Human Subjects. Geneva: Council for Organizations of Medical Sciences/WorldHealth Organization, 2002. http://www.cioms.ch/publications/layout_guide2002.pdf.

13- Cura EN , Segura EL . Qualityassurance of the serologic diagnosis of Chagas disease. Rev Panam Salud Publica 1998 3:242-8 .

14- Blejer JL, Saguier MC, Salamone HJ. Antibodies to *Trypanosomacruzi* among blood donors in Buenos Aires, Argentina. Int J Infect Dis. 2001;5(2):89-93.

15- Gürtler RE, Segura EL, Cohen JE. Congenital transmission of *Trypanosomacruzi* infection in Argentina. Emerg Infect Dis. 2003;9(1):29-32.

16- Cordova E, Boschi A, Ambrosioni J, et al. Reactivation of Chagas disease with central nervous system involvement in HIV-infected patients inArgentina, 1992-2007. Int J Infect Dis. 2008;12(6):587-92.

ACKNOWLEDGEMENTS

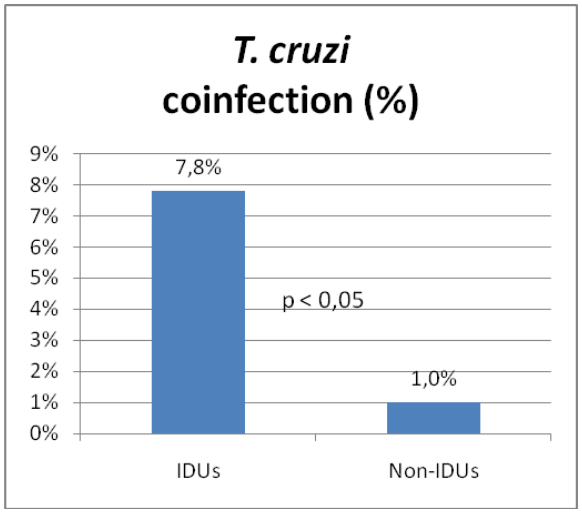
This work was partly supported by grants from Argentina's National Agency for Promotion of Science and Technology (PICT 05-33947 to LMP), the National Council for Technology and Scientific Research (PIP 6119 to GLD), and the University of Buenos Aires (UBACyT ME043 to LMP).

Table. HIV - *Tripanosoma cruzi* coinfection frequency in different populations

HIV- positive Populations	Nr studied	Positive *T.cruzi* serology*	Frequency of coinfection, %
IDUs	77	6	7.8
NICUs	28	0	0
ETS	51	2	3.9
MSM	124	0	0
Total	280	8	2.9

IDUs: intravenous drug users, NICUs: non intravenous cocaine users, MSM: men who have sex with men.

*Chagas serology was considered reactive with at least two reactive different techniques.



Legend to figure.

Figure. *T. cruzi* infection comparison between HIV positive IDUs (n = 77) and HIV Non-IDUs (n = 203)