Clinical Infectious Diseases

BRIEF REPORT

Adaptation of Chagas Disease Screening Recommendations for a Community of At-risk HIV in the United States

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Chagas disease (CD), caused by *Trypanosoma cruzi*, is underdiagnosed in the United States. Improved screening strategies are needed, particularly for people at risk for life-threatening sequelae of CD, including people with human immunodeficiency virus (HIV, PWH). Here we report results of a CD screening strategy applied at a large HIV clinic serving an at-risk population.

Keywords. Chagas disease; *Trypanosoma cruzi*; human immunodeficiency virus (HIV); acquired immunodeficiency syndrome (AIDS); screening; immunocompromised; reactivation.

BACKGROUND

Chagas disease (CD), caused by *Trypanosoma cruzi*, affects 6–7 million people in the Americas, including an estimated 300 000 US residents [1]. Transmission can be vector-borne, congenital, or via blood transfusion or organ transplant. Most US residents with CD are Latin American immigrants infected in their birth country, but rare local transmission occurs in the southern United States. Despite 1%–2% prevalence among Latin American immigrant populations [2, 3], many US providers are unfamiliar with CD.

Early CD diagnosis is essential for people with human immunodeficiency virus (HIV. PWH) due to their risk of potentially lethal *T. cruzi* reactivation. *T. cruzi* reactivation disease is an AIDS-defining opportunistic infection in endemic areas, typically manifesting in the central nervous system (CNS) and/or heart [4]. CNS reactivation carries a high mortality rate (79%–100%) that may be mitigated by prompt antiparasitic and antiretroviral therapy [5].

Early identification of coinfection is crucial but impeded by the complexity of CD diagnosis, which requires positive results by 2 distinct anti-*T. cruzi* immunoglobulin G (IgG) assays. Furthermore, weakened cellular and humoral immune responses in PWH facilitate higher parasitemia and potentially false negative serology [4, 6, 7]. Diagnosis of CD in PWH might be improved by using molecular tools.

Practical, reliable CD testing approaches are needed, particularly in populations at higher risk for severe outcomes. Our objective was to prospectively evaluate the logistics of incorporating CD screening tests for at-risk PWH in a large public healthcare system.

METHODS

Study Design and Population

This was a cross-sectional observational study of PWH presenting to Thomas Street Health Center (TSHC) in Houston, Texas, between August 2021 and June 2022. Eligible PWH were ≥18 years old and had lived ≥6 months in or were born to a mother from continental Latin America.

Enrollment Procedures

In April 2021, we invited TSHC providers to a virtual meeting to provide education about CD and posted signage in every examination room detailing screening recommendations. Enrollment began in August 2021 by approaching outpatient PWH in the laboratory waiting room or at their physician's recommendation. After written informed consent, study staff administered an electronic REDCap survey covering demographic, CD risk factor, and clinical data. We collected blood during routine phlebotomy and stored serum and whole blood aliquots at −80°C for batch analysis.

Using Epic SlicerDicer, we evaluated the number of patients screened for CD in the 3 months before the educational session (January–March) and the 3 months between the session and initiation of active patient recruitment (May–July). We compared these figures to those screened in the first 3 months of active recruitment (September-November).

Received 12 July 2023; editorial decision 30 September 2023; published online 8 October 2023

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Clinical Infectious Diseases® 2024;78(2):453–6

https://doi.org/10.1093/cid/ciad616

Study Assays

We thawed sera at room temperature and tested them for anti-*T. cruzi* IgG using 3 enzyme-linked immunoassays, Chagas' Kit (Hemagen Diagnostics), Chagatest recombinante v.3.0 (Wiener Labs), and Chagas Detect Fast (InBios International). The Hemagen and Wiener assays are Food and Drug Administration (FDA)-cleared. The InBios assay is currently for research use only but demonstrated excellent performance in a recent evaluation [8].

We extracted DNA from whole blood (Qiagen DNeasy Blood and Tissue Kit) and performed quantitative polymerase chain reaction (qPCR) using TcZ1/TcZ2 primers [9]. We tested specimens in duplicate using TaqMan® Fast Advanced Master Mix (Life Technologies) in a ViiA™ 7 Real-Time PCR system (Applied Biosystems) with appropriate controls, including RNAse P Gene (Sigma) internal amplification control.

We reported results to patients and their HIV providers within 2 weeks. For participants with any positive serological result, we requested an additional specimen for Centers for Disease Control and Prevention (CDC) confirmatory testing.

Data Analysis

We calculated descriptive statistics for each variable. We measured the frequency of CD using 95% exact binomial confidence intervals (CI).

The BCM Institutional Review Board approved this study.

RESULTS

Providers ordered 0 CD tests in the 3 months before the provider educational session, 4 tests in the 3 post-session months, and 32 tests in the first 3 months of active recruitment.

We offered enrollment to 359 PWH; 44 (12%) declined, 21 (6%) completed the enrollment survey but not *T. cruzi* testing, and 294 (82%) completed all procedures. Of those with complete data, 80.3% were male, and 65.3% were born in Mexico (Table 1). Most (93.2%) lived in their birth country for >10 years; 52.7% reported not completing high school. In total, 22.4% recognized photos of the vector but only 2.0% answered "yes" to the survey question "Do you know what Chagas disease is?" Also, 22.4% had resided in a house made of mud, adobe, sticks, or thatch, and 8.2% had received a blood transfusion. Only 1 participant reported family history of CD.

The median CD4 count was 494 cells/mL (IQR 6–2590) and 74.8% had an HIV viral load of <20 copies/mL (median <20 copies/mL, IQR 0–1 760 000). Two hundred eighty-four participants (96.6%) tested negative by all 3 serologic tests and *T. cruzi* PCR. Of the remaining 10 participants, 7 had positive results by Hemagen only, 1 by Hemagen and InBios, 1 by InBios only, and 1 by *T. cruzi* PCR only. Based on positive results by 2 serologic tests or *T. cruzi* PCR, the CD frequency was 2/294 (0.68%, 95% CI 0%–1.62%).

Participants with any positive serology result underwent a second blood draw for confirmatory testing. The median time from screening result to confirmatory test result was 177 days (IQR 52–305, range 28–425 days). Multiple factors caused delays, including participant transportation/work hour restrictions, confusion among laboratory staff regarding specimen type and submission, misplaced paperwork, and specimen rejection by CDC.

DISCUSSION

This is the first study to our knowledge to (1) apply a CD screening protocol in a US HIV clinic, (2) prospectively evaluate CD frequency in at-risk PWH using 3 distinct serologic tests, and (3) systematically evaluate at-risk PWH using *T. cruzi* PCR. We included serologic tests commercially available to US clinicians. Our major aim was to evaluate a routine CD screening algorithm in a public healthcare facility serving PWH with epidemiological risk. In doing so, we identified several crucial challenges.

First, a single provider educational session, even with testing instructions posted in examination rooms, was insufficient to boost screening. We quickly recognized the need for active recruitment. After this study, we trained the 2 TSHC nurse practitioners responsible for entry-to-care visits to ask patients whether they had spent ≥6 months in continental Latin America and, if so, to include *T. cruzi* serology in the entry-to-care laboratory panel. We believe inclusion of a CD screening test in entry-to-care laboratory panels for at-risk PWH (as often done for *Toxoplasma*) will improve screening completion and sustainability [4]. Systematic recording of birth country in the electronic medical record would enable a flag to signal need for CD screening to providers, like existing flags for vaccinations and cancer screenings.

Second, the additional blood draw for CDC confirmatory testing proved a major logistical challenge, with median interval from screen to confirmatory results of nearly six months. Consequently, our healthcare system is working to alter the CD screening order to be a reflex test, saving serum for automatic shipment to CDC if the screen is positive.

Finally, our finding of a single *T. cruzi* PCR-positive but seronegative patient (CD4 = 194, Table 1) suggests molecular testing should be considered an adjunct test for the subset of at-risk PWH with CD4 < 200. Previous studies have also reported false negative CD serology in severely immunosuppressed HIV-*T. cruzi* coinfected patients [6, 7]. *T. cruzi* infection in mice lacking mature B-cells results in increased parasitism and deficient memory T-cell generation, suggesting that B-cells may help control *T. cruzi* multiplication [10]. These

Table 1. Study Participant Demographics, Chagas Disease (CD) Risk Factors, and *T. cruzi* Test Results

	Total (N = 294)	All Tests (–) (N = 284)	Only Hemagen (+) (N = 7)	Only InBios (+) (N = 1)	Hemagen and nBios (+) (N = 1)	Only qPCR (+) (N = 1)
Age in years, median [range]	48 [19, 86]	48 [19, 74]	46 [36, 65]	48	86	47
Sex at birth (male), N (%)	236 (80.3)	230 (81.0)	5 (71.4)	1 (100)	0 (0)	0 (0)
Did not complete high school, N (%)	155 (52.7)	149 (52.5)	4 (57.1)	1 (100)	1 (100)	0 (0)
Birth country, N (%)						
Mexico	192 (65.3)	188 (66.2)	2 (28.6)	1 (100)	1 (100)	0 (0)
Honduras	39 (13.3)	35 (12.3)	3 (42.9)	0 (0)	0 (0)	1 (100)
El Salvador	30 (10.2)	28 (9.9)	2 (28.6)	0 (0)	0 (0)	0 (0)
Guatemala	13 (4.4)	13 (4.6)	0 (0)	0 (0)	0 (0)	0 (0)
United States	10 (3.4)	10 (3.5)	0 (0)	0 (0)	0 (0)	0 (0)
Colombia	4 (1.4)	4 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)
Venezuela	3 (1.0)	3 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)
Peru	2 (0.7)	2 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)
Nicaragua	1 (0.3)	1 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)
Years living in birth country before emigration, N (%)						
>10	274 (93.2)	264 (93.0)	7 (100)	1 (100)	1 (100)	1 (100)
0 to 10	10 (3.4)	10 (3.5)	0 (0)	0 (0)	0 (0)	0 (0)
Born in US	10 (3.4)	10 (3.5)	0 (0)	0 (0)	0 (0)	0 (0)
Chagas disease risk factors						
Lived in a house made of mud, adobe, sticks or thatch, N (%)	66 (22.4)	63 (22.2)	1 (14.3)	0 (0)	1 (100)	1 (100)
Report seeing a Triatomine insect, N (%)	66 (22.4)	64 (22.5)	1 (14.3)	0 (0)	0 (0)	1 (100)
History of major surgery, N (%)	57 (19.4)	56 (19.7)	0 (0)	0 (0)	1 (100)	0 (0)
History of blood transfusion, N (%)	24 (8.2)	23 (8.1)	0 (0)	0 (0)	0 (0)	1 (100)
History of blood donation, N (%)	13 (4.4)	13 (4.6)	0 (0)	0 (0)	0 (0)	0 (0)
HIV status data						
CD4 count, median [range]	494 [6, 2590]	497 [6, 2590]	367 [33, 1480]	275	543	194
HIV viral load, median [range]	<20 [<20, 1760000]	<20 [<20, 1760000]	<20 [<20, 141]	<20	<20	8440
On antiretroviral treatment, N (%)						
Yes	122 (41.5)	118 (41.5)	2 (28)	0 (0)	1 (100)	1 (100)
No	1 (0.3)	1 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)
No data/no report	171 (68.2)	165 (58.1)	5 (71)	1 (100)	0 (0)	0

Abbreviations: HIV, human immunodeficiency virus; qPCR, quantitative polymerase chain reaction; *T. cruzi*, *Trypanosoma cruzi*.

data are supported by 1 human study suggesting that chronic *T. cruzi* infection alters distribution of peripheral blood B-cell subsets involved in CD4 regulation [11]. This effect may be amplified in PWH with low CD4 counts.

Systematically screening at-risk US PWH with 1 sensitive serologic test, followed by confirmatory testing, as recommended for the US general population [1], is likely an adequate screening approach except for PWH with CD4 ≤ 200. Health centers serving PWH can maximize accuracy of CD testing and minimize cost to the clinic and patients by implementing testing criteria focused on the highest-risk patients, such as:

- Systematically screening PWH who have spent ≥6 months in continental Latin America with 1 sensitive serologic *T. cruzi* IgG test (eg, during their "entry-to-care" visit) and,
- Additionally testing at-risk PWH with CD4 < 200 who have spent ≥6 months in continental Latin America with 2 different serologic tests plus *T. cruzi* PCR. *T. cruzi* serology is free of charge at the Texas Department of State Health Services, and PCR is available at CDC.

Clearly, our sample size was insufficient to provide a robust prevalence estimate. Nevertheless, our estimate of 0.68% was consistent with figures reported in US community convenience samples [2, 3]. Our at-risk population was largely from Mexico and had a mean age of 48 years. We previously estimated that two-thirds of *T. cruzi* infected individuals in the United States are older than 50 years, and the estimated prevalence for Mexico is 0.7%, substantially lower than estimates for Central or South America [12].

CD screening is already recommended for all at-risk persons in the United States [1]. The incorporation of a sensitive *T. cruzi* serologic test into entry-to-care evaluation for at-risk PWH could establish a systematic mechanism for CD detection, educate providers and the vulnerable population they serve, and provide referral for appropriate management, with the potential to directly reduce *T. cruzi*-related morbidity and mortality.

Notes

Author Contributions. C. B., J. D. W., T. P. G., and E. H. C. devised the study. J. H. and E. H. C. created and carried out the TSHC provider CD educational session and flyers. J. H., S. L., and E. H. C. consented and enrolled study participants and collected and processed blood specimens. C. J. F. and J. D. W. set up and performed *T. cruzi* serologic assays. J. H., C. P., K. M. J., and E. H. C. set up and performed *T. cruzi* PCR. Q. Q. and H. W. performed statistical analysis. J. H., S. L., and E. H. C. drafted the manuscript. All authors edited and contributed to the content of the manuscript.

Acknowledgments. The authors greatly appreciate the support of the TSHC patient population, research coordinators Denise Flores and Jose Molina, and the TSHC nursing and laboratory staff. The contents of this manuscript do not represent the views of the U.S. Department of Veterans Affairs or the U.S. Government.

Financial support. This work was supported by a Baylor College of Medicine junior faculty seed award (granted by the Caroline Weiss Law Fund for Research in Molecular Medicine and the L.E. Gordy Cancer Research Fund to E. H .C.) and by the Texas Developmental Center for AIDS Research (grant number P30A1161943).

Potential conflicts of interest. C. B. reports royalties or licenses from Wolters Kluwer. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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