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Chagas Disease Screening Using Point-of-Care Testing in an At-Risk Obstetric Population

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Abstract. Congenital transmission is the most important mode of transmission of Chagas disease (CD) in non-endemic countries. Identifying CD in reproductive-aged women is essential to reduce the risk of transmitting the disease to their children and offer treatment to women and their children, which could cure the disease. We evaluated the use of point-of-care (POC) testing for CD in postpartum patients. In our patient population, 16.7% (23/138) tested positive by POC testing, but confirmatory testing was negative for all patients. Among those considered high risk, 30% declined participation. Our results suggest limited utility of the point-of-care test used in our study and identify an opportunity for improvement to broaden diagnostic testing options. Our study also highlights the need to develop strategies to increase subject participation in future research.

Chagas disease (CD) is a parasitic disease caused by the protozoan *Trypanosoma cruzi*; it affects more than 5.7 million people worldwide, claims 12,000 lives annually, and causes the greatest burden of disability-adjusted life years of any parasitic disease in Latin America. Approximately 1.2 million of those affected are women of childbearing age.[1,2] Chagas disease is present in rural areas in endemic countries, and due to migration of infected individuals from rural to urban areas in Latin America and international migration from endemic to non-endemic countries, CD has emerged as a public health concern in non-endemic countries, including the United States.[3,4] Approximately 23 million people living in the United States were born in CD-endemic countries, and an estimated > 300,000 people living in the United States are infected with CD.[4] Most individuals infected are asymptomatic and unaware, making CD challenging to diagnose and treat. For patients with chronic, untreated CD, 20–30% will ultimately progress to the "determinate" disease form, characterized by irreversible heart and/or gastrointestinal disease with high morbidity and mortality. Diagnosis in the asymptomatic phase is essential to avoid long-term consequences.

In endemic countries, most of the transmission is vector borne; however, 22% of new Chagas diagnoses are from congenital transmission.[2] In non-endemic countries, congenital transmission is most common, and therefore the most important mode of transmission.[5] In addition, mothers who do not know they were infected congenitally can pass the disease to their children.[5] It is estimated that 40,000 reproductive-aged women are infected with *T. cruzi* in the United States.[6] Women with CD transmit the disease to their babies during pregnancy at a rate of 1–5%, resulting in congenital Chagas.[7,8] These rates are based on screening based on maternal history in research studies and are likely slightly underestimated. In public health settings, screening strategies can be based on testing infants based on maternal history or on testing infants based on symptoms, the latter being even more likely to miss cases, as babies with congenital CD are often asymptomatic. In addition, some neonatal screening tests have low sensitivity or limited availability in resource-limited settings, and many patients do not follow-up for completion of testing.[9] Symptomatic infants can have low birth weight, anemia, hepatosplenomegaly, or severe infections that can lead to death. Infants with congenital CD who survive the acute phase are presumed to have the same lifetime risk of the "determinate" form (20–30%) as adults with CD.[8,10] They also risk transmitting the disease through blood or organ donation or to future children, sustaining the infection across generations in the absence of the vector. Diagnosing a mother or infant with congenital Chagas increases the likelihood that other family members will be diagnosed and offered early treatment.[11]

Treatment is recommended for reproductive-aged women in the absence of Chagas cardiomyopathy and children younger than 18 years. Benznidazole and nifurtimox are the antitrypanosomal drugs used to treat CD. Benznidazole and nifurtimox are approved by the Food and Drug Administration (FDA) for use in children aged 2–12 years and those younger than 18 years, respectively. Both are available off-label for treatment in adults through their distributors.[6,12,13] Women with CD should not be treated during pregnancy or while breastfeeding because of concerns about medication safety; treatment is recommended after completion of breastfeeding.[11,14] Treatment in reproductive-aged women can likely decrease the risk of disease transmission to future children.[15] Infants infected congenitally can achieve a > 90% cure if treated within the first year of life.[16,17]

Screening for CD in the United States in at-risk reproductive-aged women is currently recommended by the CDC but is not routinely performed because of lack of physician and patient knowledge and dearth of healthcare resources in the at-risk population.[11,18,19] There are insufficient data in the United States regarding the prevalence of CD; thus, the public health burden remains unclear.

This study sought to examine the feasibility of screening for CD in an at-risk obstetric population in the United States through development of a screening algorithm using point-of-care (POC) testing and to identify the prevalence of CD in our hospital. Our study was conducted from January to November 2019, at Grady Health System in Atlanta, GA, among postpartum women. The study was approved by Emory School of Medicine and Grady Health System's Institutional Review

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Boards and was funded by the Emory Medical Care Foundation. We chose to approach postpartum patients as, although women should not be treated for CD while pregnant or breastfeeding, many women only receive health care during pregnancy. Thus, this provides a unique window for healthcare access for women and their families, especially given the higher rate of successful treatment among younger children. Our projected sample size at the start of the study was 456 patients based on the desired precision of a 95% CI using an estimated 1.2% ± 1% prevalence of Chagas from previous studies.[20] Approximately 3,100 deliveries occur in our hospital annually, of which 20% meet eligibility requirements; thus, we felt we could meet our projected sample size in 1 year of testing.

Study staff approached postpartum patients, and those who met the inclusion criteria (they or their mothers were born in Mexico, or Central or South America or had a family history of CD) were invited to participate in the study using a standardized study script. Inclusion criteria were chosen to select women with the highest probability of risk for CD. Those who agreed to participate and consented were screened for CD and completed a questionnaire to collect demographic data and information on risk factors including amount of time lived outside the United States, which countries, and characteristics of their housing while living outside the United States.

Diagnosis of CD requires two different format assays with different antigen preparations to be positive.[11,21] We used POC IgG technology (InBios Chagas Detect Plus, InBios International, Inc., Seattle, WA) as the first step of the two-step confirmation to improve diagnosis because testing could be interpreted at bedside and confirmatory testing drawn in the same clinical episode. This is the first POC IgG serology test, cleared by the FDA for use in 2016, with a reported > 95% sensitivity and specificity in endemic populations with a high prevalence of CD and > 95% sensitivity but a lower specificity (88–92%) in non-endemic populations as shown in a recent study comparing CD testing modalities in U.S. blood donors.[21–23] Research staff were trained on correct POC test administration per manufacturer instructions and were directly observed in the first week of the study. If a patient had a positive POC test, a venous sample was drawn and sent to Quest laboratories for confirmation via Hemagen ELISA testing for antibodies to *T. cruzi*. This was the first study in the United States examining POC testing for CD screening in pregnancy.

Once initiated, we noted several patients had positive POC tests and negative Quest confirmatory test results. Most of our positive POC tests were faint positives; thus, we contacted InBios to review our test results including photographs of our positive tests, for guidance. The recommendations from InBios were to report even faintly positive tests as positive. Given these recommendations, we consulted with Chagas experts at the CDC, and chose to modify the study protocol to conduct two confirmatory tests, CDC and Quest, after a positive POC test. The CDC uses serology to perform two tests, a recombinant antigen ELISA, Weiner Chagatest ELISA recombinant 3.0, and trypomastigote-excreted secreted antigen immunoblot. If these results are discordant, an immunofluorescent antibody assay is used as a "tie breaker."

We intended to continue our study for 12 months, but because of unanticipated lack of accuracy of the POC test, we stopped our study after the 11 month of enrollment. At study completion, 1,255 patients were approached, and of these, 196 were found to be eligible based on risk factors. Patients were ineligible largely because of not being born in or having lived in an endemic country. One hundred thirty-eight of the eligible patients (70%) consented for testing and were enrolled in the study. The demographic characteristics of these patients are shown in Table 1. Among our study cohort, 23 (16.7%) had positive POC tests; Quest and CDC confirmatory testing was completed for 21 and 18 patients, respectively. All Quest and CDC confirmatory testing were negative. These results are shown in Figure 1. Confirmatory testing was not completed for two patients because the hospital laboratory lost the blood samples drawn following positive POC testing. Because we had no positive confirmatory tests among our study cohort and did not conduct confirmatory tests for

TABLE 1
Demographic and socioeconomic profile of postpartum women in the study population

Demographics	Total study population (N = 138), n (%)
Age (years)	
< 21	19 (13.8)
21–34	79 (57.3)
> 34	40 (29.0)
Born in Latin America	124 (89.9)
Birth country	
Colombia	2 (1.5)
El Salvador	5 (3.7)
Guatemala	29 (21.2)
Honduras	18 (13.1)
Mexico	64 (46.7)
Peru	2 (1.5)
United States	14 (10.2)
Venezuela	3 (2.2)
Years lived in Latin America	
0	4 (3.0)
1–5	9 (6.5)
6–10	13 (9.4)
11–15	9 (6.5)
16–20	53 (38.4)
21–25	27 (19.6)
26–30	17 (12.3)
31–35	6 (4.4)
Years lived in the United States	
0	6 (4.4)
1–5	36 (26.1)
6–10	16 (11.6)
11–15	28 (20.3)
16–20	42 (30.4)
21–25	10 (7.3)
Education level	
Less than high school	73 (52.9)
High school graduate	50 (36.2)
Some college, no degree	10 (7.3)
Associate's degree	1 (0.7)
Bachelor's degree	2 (1.5)
Graduate or professional degree	2 (1.5)
Marital status	
Married	44 (31.9)
Single	94 (68.1)
Employment status	
Full time	5 (3.6)
Part time	6 (4.4)
Unemployed	127 (92.0)
Insurance status	
Medicaid	75 (54.4)
Private insurance	4 (3.0)
Medicare	34 (24.6)
Uninsured	25 (18.1)
Lived in rural Latin America > 6 months	115 (83.3)
Prior knowledge of Chagas disease	
Yes	28 (20.3)
No	110 (79.7)

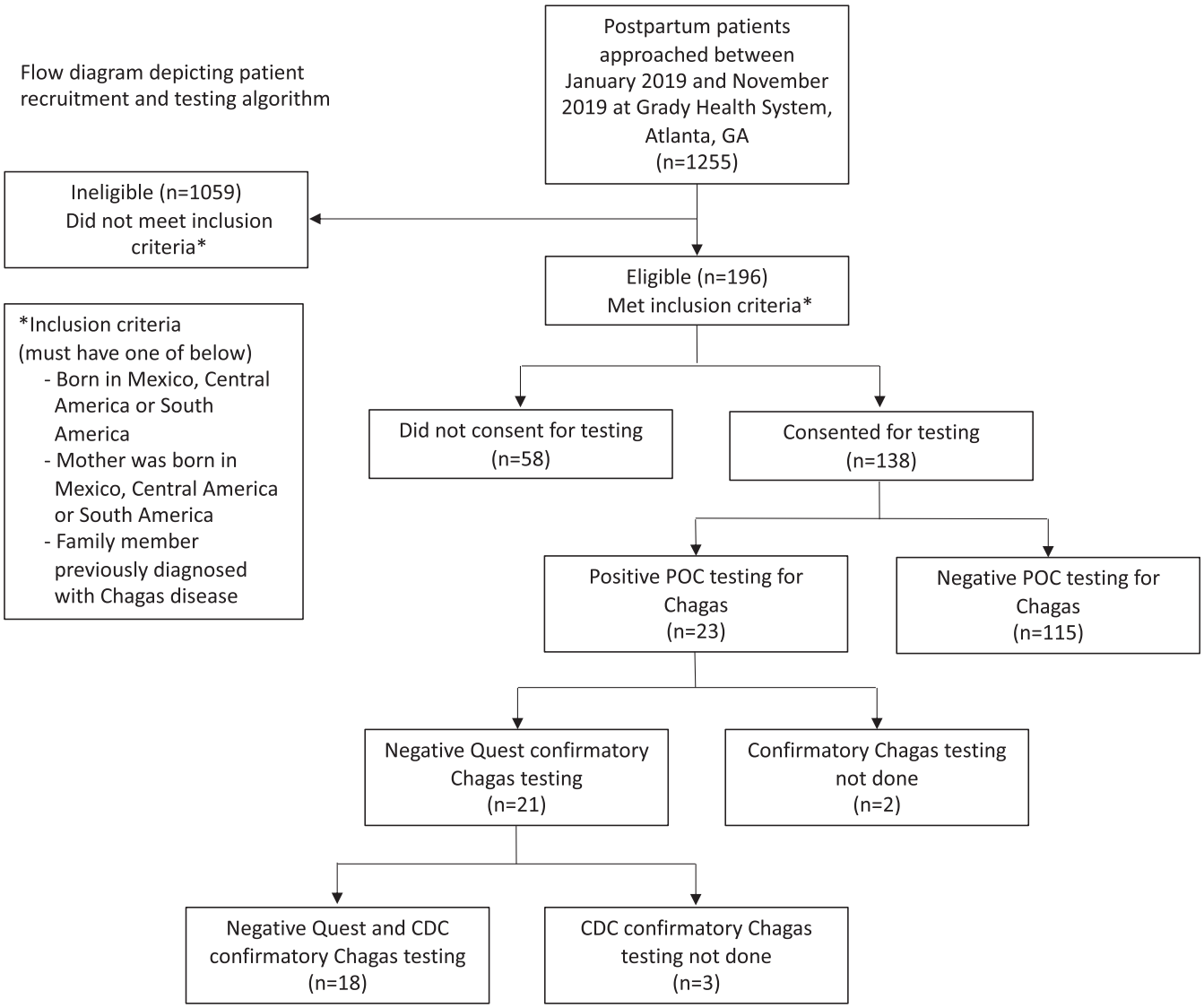

FIGURE 1. Flow diagram depicting patient recruitment and testing algorithm.

patients with negative POC tests, we are not able to calculate diagnostic accuracy measures in our population. Comparing patients with positive and negative POC testing, there were no significant differences in risk factors between the two groups.

We encountered multiple challenges while implementing this study. First, an unexpectedly high number (30%) of patients who were eligible for the study did not consent to participate, contrary to our anticipated consent rate of 90%. We hired native Spanish speakers as our research staff and purposely did not include any questions relating to patients' legal status in an effort to make patients feel more comfortable during what we anticipated to be a vulnerable time. Research staff also provided basic patient education about CD while conducting the survey in an attempt to spread knowledge and awareness of the disease. Lower rates of consent could be attributed to a number of factors, including stigma surrounding a disease that disproportionally affects the poor and the current political climate surrounding immigrant populations. Unfortunately, reasons for refusal were not collected, and without this information, it is difficult to tell if systematic bias was introduced into the study. This was a missed opportunity that could have given important information leading to improved study design in the future. We feel that making efforts to integrate screening programs into routine health screenings, for example, into yearly well woman examinations or health fairs, could help to destigmatize CD testing and improve consent rates as compared with testing in a research setting. Utilization of community health workers to provide enhanced patient education at the time of testing is another strategy that could be beneficial.

After the first few weeks of testing, the protocol was modified to perform two forms of confirmatory testing. Although patients with positive POC testing before this protocol change were contacted to return for a second confirmatory test, this proved to be challenging and often unsuccessful. We also attempted to contact patients with lost samples, but were unsuccessful and ultimately did not have confirmatory results for two patients. The patient population at risk for CD in the United States has baseline risk factors for difficulty obtaining healthcare services, and we believe this impacted their ability

to follow-up.[24] Although previous studies reported high sensitivity and specificity of this POC test (approximately 95%) in endemic areas, our results did not echo these findings. We anticipated having a low prevalence of CD in our population, and although the prevalence should not affect the test's specificity, reported specificity for the test has been variable between non-endemic and endemic populations.[23] Most of our positive POC tests were weakly positive, and although the test's manufacturer advised to report these as positive, reading rapid tests is subjective, and this subjectivity particularly in the case of weak positive results could have impacted our results. Specificity of the POC test based on our study was 83%, 95% CI: 77.1–89.6 (115/138) if Quest and/or CDC testing is treated as the gold standard, and the two specimens with no confirmatory testing were assumed to be negative. Although this specificity estimate is lower than estimates in previous studies that examine this POC test in U.S. populations, it does overlap with the 95% CIs.[23] Future study design needs to focus on making data-based sample size calculations to ensure that enough patients are tested to capture true positives. We used an estimated prevalence rate of 1.2% for our sample size calculation, but other studies in U.S. populations have shown lower prevalence of Chagas.[25] Given the number of false positives with no true positives obtained, we concluded that there is a need for development of POC tests for CD with higher specificity to use POC testing as a screening tool in our population.

Although we set out to demonstrate the success of an in-hospital screening algorithm for CD, our challenges demonstrate the limitations of our current approach. Our research highlights the need for improved availability and quality of screening tests. In addition, research is needed on factors that impact willingness to participate in screening and how to best reach at-risk populations, perhaps by combining screening programs for CD with screening for more common conditions to maximize screening and benefit to patients. Globally, CD remains an underlying public health challenge, and future research should aim to identify effective strategies in non-endemic countries for capturing patients before disease progression and irreversible health consequences.

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