ORIGINAL ARTICLE

Randomized Trial of Benznidazole for Chronic Chagas' Cardiomyopathy

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ABSTRACT

BACKGROUND

The role of trypanocidal therapy in patients with established Chagas' cardiomyopathy is unproven.

METHODS

We conducted a prospective, multicenter, randomized study involving 2854 patients with Chagas' cardiomyopathy who received benznidazole or placebo for up to 80 days and were followed for a mean of 5.4 years. The primary outcome in the time-to-event analysis was the first event of any of the components of the composite outcome of death, resuscitated cardiac arrest, sustained ventricular tachycardia, insertion of a pacemaker or implantable cardioverter–defibrillator, cardiac transplantation, new heart failure, stroke, or other thromboembolic event.

RESULTS

The primary outcome occurred in 394 patients (27.5%) in the benznidazole group and in 414 (29.1%) in the placebo group (hazard ratio, 0.93; 95% confidence interval [CI], 0.81 to 1.07; P=0.31). At baseline, a polymerase-chain-reaction (PCR) assay was performed on blood samples obtained from 1896 patients; 60.5% had positive results for *Trypanosoma cruzi* on PCR. The rates of conversion to negative PCR results (PCR conversion) were 66.2% in the benznidazole group and 33.5% in the placebo group at the end of treatment, 55.4% and 35.3%, respectively, at 2 years, and 46.7% and 33.1%, respectively, at 5 years or more (P<0.001 for all comparisons). The effect of treatment on PCR conversion varied according to geographic region: in Brazil, the odds ratio for PCR conversion was 3.03 (95% CI, 2.12 to 4.34) at 2 years and 1.87 (95% CI, 1.33 to 2.63) at 5 or more years; in Colombia and El Salvador, the odds ratio was 1.33 (95% CI, 0.90 to 1.98) at 2 years and 0.96 (95% CI, 0.63 to 1.45) at 5 or more years; and in Argentina and Bolivia, the odds ratio was 2.63 (95% CI, 1.89 to 3.66) at 2 years and 2.79 (95% CI, 1.99 to 3.92) at 5 or more years (P<0.001 for interaction). However, the rates of PCR conversion did not correspond to effects on clinical outcome (P=0.16 for interaction).

CONCLUSIONS

Trypanocidal therapy with benznidazole in patients with established Chagas' cardiomyopathy significantly reduced serum parasite detection but did not significantly reduce cardiac clinical deterioration through 5 years of follow-up. (Funded by the Population Health Research Institute and others; ClinicalTrials.gov number, NCT00123916; Current Controlled Trials number, ISRCTN13967269.)

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Chagas' disease is the third most common parasitic disease globally, after malaria and schistosomiasis.[1] Chagas' cardiomyopathy is the most common form of nonischemic cardiomyopathy and one of the leading causes of complications and death in Latin America.[2] An estimated 6 million to 7 million persons are infected, and 36,800 new cases occur each year. Chagas' cardiomyopathy develops in approximately 25% of patients infected with *Trypanosoma cruzi*.[3-5]

Chagas' disease has two phases: acute and chronic. Acute infection is usually a self-limited febrile illness.[6] In the chronic phase, cardiac or digestive complications develop in approximately one third of patients two or three decades after the acute infection.[7] Chronic Chagas' cardiomyopathy is associated with malignant arrhythmias, conduction disturbances, heart failure, and pulmonary and systemic embolism[2,7] and is associated with an annual mortality of approximately 4% among patients who are followed in outpatient clinics.[8]

T. cruzi causes an acute disease, which can be cured with trypanocidal treatment.[6] However, in chronic cardiomyopathy, the role of the parasite is debated and the effect of trypanocidal treatment is unclear.[9,10] In some previous studies, autoimmune mechanisms were implicated as potential causes of late cardiac injury[11-14] because of the apparent absence of parasites in the cardiac inflammatory lesions on classic histologic analysis and the occurrence of autoimmune responses related to polyclonal activation, molecular self-mimicry by parasite antigens, or cryptic epitopes shared by the host and parasites.[2,14]

However, the identification of *T. cruzi* antigens in inflamed myocardium with the use of sensitive techniques, such as immunohistochemical analysis and polymerase-chain-reaction (PCR) assay, suggests that parasite persistence may be an important factor that, in conjunction with individual host factors, triggers the inflammatory process.[14-16] In assessing whether trypanocidal therapy prevents or reduces cardiac disease, experimental models of chronic Chagas' infection have shown that trypanocidal therapy attenuates the pathologic consequences by reducing the parasite burden.[17,18] A few small observational and randomized studies involving patients with chronic Chagas' disease have shown that benznidazole reduces the circulating parasite load, enhances seroconversion, and may halt the progression of cardiomyopathy.[19-22]

We designed the Benznidazole Evaluation for Interrupting Trypanosomiasis (BENEFIT) trial to evaluate the efficacy and safety of benznidazole, as compared with placebo, in reducing clinical outcomes among patients with chronic Chagas' cardiomyopathy.

METHODS

STUDY DESIGN

From 2004 through 2011, we conducted a randomized, double-blind, placebo-controlled trial in which we administered either benznidazole or matching placebo orally in 2854 patients for 40 to 80 days at 49 centers in Argentina, Bolivia, Brazil, Colombia, and El Salvador. The Population Health Research Institute at Hamilton Health Sciences and McMaster University in Hamilton, Ontario, Canada, and Dante Pazzanese Research Institute in São Paulo coordinated the trial. Ethics approvals were obtained at the coordinating and clinical centers. All the patients provided written informed consent. Full details are provided in the protocol, which is available with the full text of this article at NEJM.org.

STUDY POPULATION

Inclusion and exclusion criteria have been reported previously.[23] Eligible patients were between 18 and 75 years of age, had at least two positive serologic tests for *T. cruzi*, and had evidence of cardiomyopathy. (Details are provided in the Supplementary Appendix, available at NEJM.org.) Patients who fulfilled the enrollment criteria were randomly assigned to receive benznidazole or matching placebo. Because of logistic constraints related to the production of benznidazole, the standard regimen (5 mg per kilogram of body weight per day for 60 days) was modified in February 2009 to the administration of a fixed dose of 300 mg per day and a variable duration of therapy (between 40 and 80 days) on the basis of the patient's weight, thereby preserving the total dose. The drug and placebo were purchased at full cost (initially from Roche and later from LAFEPE) by the Population Health Research Institute and Fundação de Amparo ao Ensino, Pesquisa e Assistência, Faculdade de Medicina de Ribeirão Preto, University of São Paulo, São Paulo. (We performed the proper

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sensitivity analysis on all outcomes including safety, and there were no differences among the batches.)

STUDY PROCEDURES

Patients were evaluated at 11 days and 21 days, at the end of treatment, at 6 months, and then annually until the end of the study. Adverse events, results of liver-function testing, and findings on 12-lead electrocardiography were recorded at baseline and during each follow-up visit during the treatment period. A 10-ml blood sample to be used for a qualitative conventional PCR assay for the detection of circulating *T. cruzi* kinetoplast DNA (kDNA) by means of an internationally validated method[24] was obtained from 1896 patients at baseline (after funding was obtained for this purpose), from 1618 patients at the end of treatment, from 1530 patients at 2 years, and from 1487 patients at the final follow-up visit. All negative kDNA results on PCR assay were amplified with human gene-specific primers, which minimized the possibility of false negative results.[25] Methods were standardized across the three core laboratories. (For details, see the Methods section in the Supplementary Appendix.)

STUDY OUTCOMES

The primary study outcome in the time-to-event analysis was the first occurrence of death, resuscitated cardiac arrest, insertion of a pacemaker or an implantable cardioverter–defibrillator, sustained ventricular tachycardia, cardiac transplantation, new heart failure, stroke or transient ischemic attack, or a systemic or pulmonary thromboembolic event. An event adjudication committee reviewed all cardiovascular outcomes in a blinded fashion.

Secondary outcomes included the response to treatment on the basis of results on PCR assay overall and according to geographic region corresponding to the common prevalent *T. cruzi* discrete typing units (i.e., genetic subtypes): *T. cruzi* I in Colombia and El Salvador, *T. cruzi* II in Brazil, and *T. cruzi* V and VI in both Argentina and Bolivia.[26-28]

STATISTICAL ANALYSIS

We determined that the enrollment of 2800 patients would provide a power of 90% to detect a relative risk reduction of 26% in the composite outcome in the benznidazole group after a mean of 5 years of follow-up, at a two-sided alpha level of 0.05. This calculation was based on an expected event rate of 8% per year in the control group, an expected rate of nonadherence in the benznidazole group of 17%, and a 3% rate of loss to follow-up. All the patients who underwent randomization were included in the analyses.

In the primary time-to-event analysis, we compared the rate of the first occurrence of any component of the primary composite outcome between the two groups. Data for 14 patients who were lost to follow-up were censored at the last observation.

We assessed the proportionality assumption of the Cox regression model by including a time–treatment interaction term in the Cox model. We used the Cox proportional-hazards model to investigate the influence of important confounders and prognostic factors.

We used generalized estimating equations to determine the proportion of patients with conversion to negative results for *T. cruzi* on PCR in the two study groups to account for correlation in repeated measures in the same patient with an unstructured correlation matrix.[29] The fixed effects that were included in the model were group, time, and the between-group interaction, and time was assessed with the patient specified as a random variable. Robust variance estimators were used. Treatment effects are summarized by odds ratios with 95% confidence intervals, provided by the logit as the link function.

Categorical variables are presented as numbers and percentages, with P values for the between-group comparisons calculated by means of chi-square tests or Fisher's exact tests in cases in which expected numbers were less than five per group. For continuous variables, data were summarized as means and standard deviations, and groups were compared with the use of t-tests. Nonnormally distributed variables are presented as medians and interquartile ranges, with a comparison of groups by means of Wilcoxon rank-sum tests. Two-sided P values of less than 0.05 were considered to indicate statistical significance.

We explored the consistency of treatment effects in the 12 prespecified subgroups using tests of interaction in Cox regression models for the primary outcome. We used the same generalized estimating equations to determine the proportion of patients with conversion to negative results

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for *T. cruzi* on PCR as we used for the overall results by including two-way and three-way interaction terms. All analyses were performed with the use of SAS software for the UNIX operating system, version 9.2 (SAS Institute), and graphics were produced with the use of TIBCO Spotfire S-Plus for Windows, version 8.2.

RESULTS

STUDY PATIENTS

From November 2004 through October 2011, a total of 2854 patients underwent randomization, with 1431 assigned to the benznidazole group and 1423 to the placebo group (Fig. S1 in the Supplementary Appendix). The majority of the patients were recruited in Brazil (1358 patients), followed by Argentina (559), Colombia (502), Bolivia (357), and El Salvador (78). Most patients (97%) had New York Heart Association (NYHA) class I or II heart failure, and the mean ejection fraction was 55%. The mean (±SD) age was 55±11 years. Baseline characteristics were well balanced between the two study groups (Table 1). Follow-up data were available for 100% of the patients at 1 year, for 99% at 2 years, and for 99.5% at 7 years. A total of 14 patients (0.5%) were lost to follow-up at the end of the study.

PRIMARY OUTCOME

The primary outcome occurred in 394 patients (27.5%) in the benznidazole group and 414 patients (29.1%) in the placebo group (unadjusted hazard ratio, 0.93; 95% confidence interval [CI] 0.81 to 1.07; P=0.31; adjusted hazard ratio, 0.92; 95% CI, 0.81 to 1.06; P=0.26) (Table 2 and Fig. 1). No significant between-group differences were observed in any component of the primary outcome. In a per-protocol analysis of data from patients who took at least 75% of the target dose, the hazard ratio was 0.90 (95% CI, 0.78 to 1.04; P=0.16).

SECONDARY OUTCOMES

Of the 1896 patients who provided a blood sample for PCR assay before randomization, results were positive in 59.5% of patients in the benznidazole group and in 61.7% of those in the placebo group. Among those with positive results at baseline, PCR conversion rates were 66.2% in the benznidazole group and 33.5% in the placebo group at the end of treatment, 55.4% and 35.3%, respectively, at 2 years, and 46.7% and 33.1%, respectively, at 5 years or more (P<0.001 for all comparisons). Repeated-measures analysis of results on PCR assay showed significantly higher conversion rates in the benznidazole group at all time points, but the relative efficacy appeared to decline over time (odds ratio at the end of treatment, 2.75 [95% CI, 2.24 to 3.36]; odds ratio at 2 years, 2.26 [95% CI, 1.85 to 2.77]; and odds ratio at 5 years or more, 1.78 [95% CI, 1.45 to 2.18]) (P<0.001 for all comparisons). The rate of persistently negative results on PCR among those who had negative PCR results at baseline was also significantly higher in the benznidazole group than in the placebo group (70.3% vs. 59.4% at the end of treatment, P=0.005; 64% vs. 54.1% at 2 years, P=0.03; and 63% vs. 52.4% at 5 years or more, P=0.02). New abnormalities on electrocardiography occurred in 36.7% of the patients in the benznidazole group and in 35.6% of those in the placebo group at 2 years (odds ratio, 1.05; 95% CI, 0.89 to 1.24) and in 38.2% and 38.2%, respectively, at 5 years or more (odds ratio, 1.00; 95% CI, 0.84 to 1.19).

SUBGROUP ANALYSIS

The patients' PCR status at baseline did not have a significant effect on the primary clinical outcome; among patients with PCR-positive status, 24.6% of the patients in the benznidazole group and 26.9% of those in the placebo group had a primary clinical event (hazard ratio, 0.91; 95% CI, 0.73 to 1.14), and among those with PCR-negative status, 23.7% of the patients in the benznidazole group and 25.3% of those in the placebo group had a primary clinical event (hazard ratio, 0.92; 95% CI, 0.69 to 1.23) (P=0.96 for interaction) (Fig. 2).

T. cruzi genotypes can differ among geographic locations, which can affect patients' responses to benznidazole.[26-30] Although we did not perform genotype analyses, on the basis of previous studies, we analyzed our population in the following subgroups: Colombia and El Salvador (where *T. cruzi* I is most prevalent), Brazil (where *T. cruzi* II is most prevalent), and Argentina and Bolivia (where *T. cruzi* V and VI are most prevalent). Rates of PCR conversion according to region were lowest in Colombia and El Salvador

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Table 1. Characteristics of the Patients at Baseline.*

Characteristic	Benznidazole (N=1431)	Placebo (N=1423)
Age — yr	55.4±10.7	55.2±11.2
Male sex — no. (%)	726 (50.7)	682 (47.9)
Any abnormal result on electrocardiography — no./total no. (%)	1335/1431 (93.3)	1348/1423 (94.7)
Right bundle-branch block or left anterior fascicular block		
Right bundle-branch block	691/1335 (51.8)	702/1348 (52.1)
Left anterior fascicular block	643/1335 (48.2)	618/1348 (45.8)
Both conditions	465/1335 (34.8)	442/1348 (32.8)
Sinus bradycardia <50 beats/min	159/1335 (11.9)	161/1348 (11.9)
Low-voltage QRS	178/1335 (13.3)	163/1348 (12.1)
ST–T wave changes	393/1335 (29.4)	405/1348 (30.0)
Q waves	45/1335 (3.4)	24/1348 (1.8)
Atrial fibrillation — no. (%)	107 (7.5)	90 (6.3)
Complex ventricular arrhythmia — no. (%)	221 (15.4)	189 (13.3)
Resuscitated cardiac arrest — no. (%)	19 (1.3)	16 (1.1)
Previous heart failure — no. (%)	142 (9.9)	128 (9.0)
New York Heart Association class — no./total no. (%)		
Patients with score	1431/1431 (100)	1421/1423 (99.9)
Class I	1065/1431 (74.4)	1045/1421 (73.5)
Class II	327/1431 (22.9)	343/1421 (24.1)
Class III	39/1431 (2.7)	33/1421 (2.3)
Pacemaker — no. (%)	205 (14.3)	198 (13.9)
Implantable cardioverter–defibrillator — no. (%)	39 (2.7)	31 (2.2)
Stroke or transient ischemic attack — no. (%)	61 (4.3)	62 (4.4)
Systemic or pulmonary embolism — no. (%)	7 (0.5)	11 (0.8)
Echocardiography performed <1 yr before randomization		
Patients with results — no. (%)	1126 (78.7)	1121 (78.8)
Left ventricular ejection fraction		
Mean — %	54.4±14.8	54.6±14.6
Value <40% — no./total no. (%)	200/1126 (17.8)	189/1121 (16.9)
Wall-motion abnormality — no./total no. (%)	431/1126 (38.3)	422/1121 (37.6)
Medication — no. (%)		
Diuretic	435 (30.4)	425 (29.9)
Spironolactone	241 (16.8)	237 (16.7)
ACE inhibitor or ARB	710 (49.6)	700 (49.2)
Digoxin	162 (11.3)	147 (10.3)
Beta-blocker	444 (31.0)	431 (30.3)
Amiodarone	284 (19.8)	267 (18.8)

* Plus–minus values are means ±SD. There were no significant differences between the groups except for Q waves (P=0.009). ACE denotes angiotensin-converting enzyme, and ARB angiotensin-receptor blocker.

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Table 2. Primary Outcome and Its Components, Hospitalizations, and Deaths.

Outcome	Benznidazole (N=1431)	Placebo (N=1423)	Hazard Ratio (95% CI)	P Value
	number (percent)			
Primary composite outcome	394 (27.5)	414 (29.1)	0.93 (0.81–1.07)	0.31
Death	246 (17.2)	257 (18.1)	0.95 (0.79–1.13)	—
Resuscitated cardiac arrest	10 (0.7)	17 (1.2)	0.58 (0.27–1.28)	—
Sustained ventricular tachycardia	33 (2.3)	41 (2.9)	0.80 (0.50–1.26)	—
New or worsening heart failure	109 (7.6)	122 (8.6)	0.88 (0.68–1.14)	—
Pacemaker or implantable cardioverter–defibrillator	109 (7.6)	125 (8.8)	0.86 (0.66–1.11)	—
Stroke or transient ischemic attack, systemic embolism, or pulmonary embolism	54 (3.8)	61 (4.3)	0.88 (0.61–1.26)	—
Cardiac transplantation	3 (0.2)	9 (0.6)	0.33 (0.09–1.22)	—
Hospitalization				
Any	358 (25.0)	397 (27.9)	0.89 (0.77–1.03)	0.11
For cardiovascular causes	242 (16.9)	286 (20.1)	0.83 (0.70–0.98)	0.03
Death from cardiovascular causes	194 (13.6)	203 (14.3)	0.94 (0.77–1.15)	0.55
Death from or hospitalization for cardiovascular causes	348 (24.3)	380 (26.7)	0.89 (0.77–1.03)	0.13

(odds ratio at the end of treatment, 1.15; 95% CI, 0.81 to 1.62; odds ratio at 2 years, 1.33; 95% CI, 0.90 to 1.98; and odds ratio at 5 or more years, 0.96; 95% CI, 0.63 to 1.45). Conversion rates were higher in Brazil (odds ratio at the end of treatment, 7.20 [95% CI, 4.53 to 11.4]; odds ratio at 2 years, 3.03 [95% CI, 2.12 to 4.34]; and odds ratio at 5 or more years, 1.87 [95% CI, 1.33 to 2.63]) and in Argentina and Bolivia (odds ratio at the end of treatment, 3.32 [95% CI, 2.43 to 4.54]; odds ratio at 2 years, 2.63 [95% CI, 1.89 to 3.66]; and odds ratio at 5 or more years, 2.79 [95% CI, 1.99 to 3.92]) (P<0.001 for interaction). However, the effect on the primary clinical outcome was not statistically heterogeneous, with rates of 24.1% in the benznidazole group and 25.6% in the placebo group in Colombia and El Salvador (hazard ratio, 0.92; 95% CI, 0.66 to 1.27); 33.2% and 37.6%, respectively, in Brazil (hazard ratio, 0.85; 95% CI, 0.71 to 1.02); and 21.4% and 18.5%, respectively, in Argentina and Bolivia (hazard ratio, 1.18; 95% CI, 0.88 to 1.58) (P=0.16 for interaction) (Fig. 2).

There was no significant difference in treatment response on the basis of individual markers of clinical severity, including NYHA class, cardiothoracic ratio of more than 0.5, segmental or global wall-motion abnormalities, low QRS voltage, left ventricular end diastolic diameter of more than 5.0 mm, or left ventricular ejection fraction of less than 40%, or on the basis of sex or age (Fig. 2).

The treatment response on the basis of PCR conversion for the 12 subgroups that were analyzed indicated a significant difference in response according to country (P<0.001 for interaction) (Fig. 3). No other significant interactions were observed on the basis of PCR conversion.

STUDY-DRUG ADHERENCE AND SAFETY

Adherence to the study-drug protocol (receipt of ≥75% of the target dose) was reported in 84% of the patients in the benznidazole group and in 94% of those in the placebo group. The rate of drug interruption because of an adverse event was significantly higher in the benznidazole group than in the placebo group (23.9% vs. 9.5%, P<0.001). Cutaneous rash, gastrointestinal symptoms, and nervous system disorders were the most common reasons for drug interruptions (Table 3).

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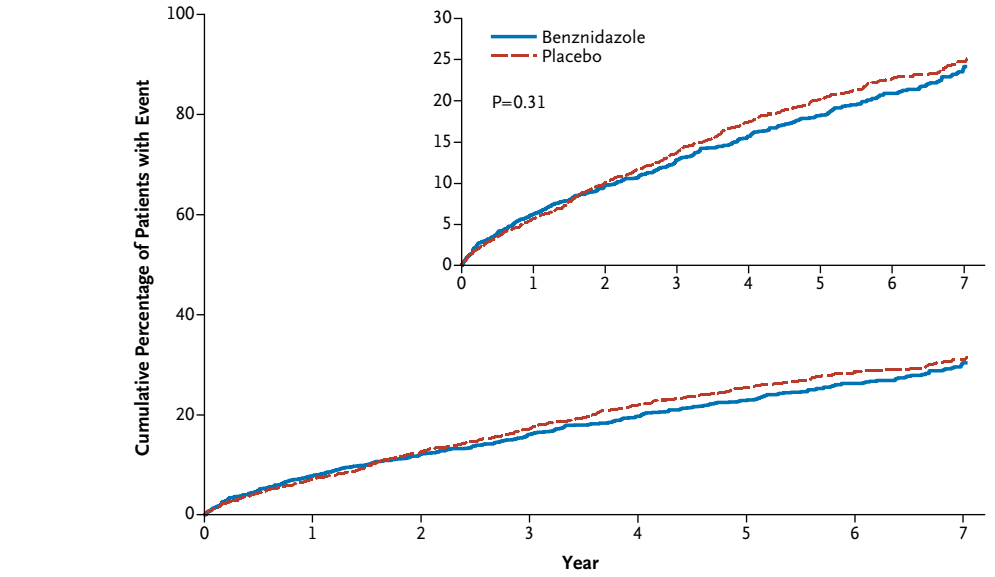

Figure 1. Primary Composite Outcome during 7 Years of Follow-up.

Shown is the rate of the primary composite outcome (death, resuscitated cardiac arrest, sustained ventricular tachycardia, insertion of a pacemaker or implantable cardioverter–defibrillator, cardiac transplantation, new heart failure, stroke or transient ischemic attack, or other thromboembolic event) among patients in the benznidazole group and the placebo group during a 7-year period (hazard ratio in the benznidazole group, 0.93; 95% CI, 0.81 to 1.07; P=0.31 by the log-rank test). The inset shows the same data on an enlarged y axis.

DISCUSSION

Benznidazole did not significantly reduce the rate of the primary clinical outcome, despite reductions in the parasite detection in serum samples. Rates of conversion to negative PCR results varied significantly according to geographic location, but the difference in rates of conversion did not correspond to a difference in the rates of clinical outcomes. The effects of benznidazole on both clinical outcomes and rates of conversion to negative PCR results did not vary according to disease severity.

The role of treatment in patients with chronic Chagas' disease and the effect of such treatment on the progression of the disease are unclear, since data have been reported only from observational and small, randomized studies.[19-22,30,31] A meta-analysis that combined data from both observational cohorts and small, randomized trials showed that benznidazole had significant activity against *T. cruzi*, as assessed by either seroconversion or significant reductions in antibody titers.[32] This meta-analysis included nine studies (of which only three, involving a total of 285 patients, were randomized trials, two of which involved children) focusing on chronic Chagas' infection with no evidence of cardiomyopathy. The use of benznidazole, as compared with placebo or no treatment, increased the rate of favorable response, which was defined as negative serologic results or xenodiagnosis (global odds ratio, 18.8; 95% CI, 5.2 to 68.3). In an observational study, Viotti et al.[20] found a significantly lower risk of clinical events (including a change in the Kuschnir classification[33]) in patients treated with benznidazole than in the untreated group (12 of 283 patients [4.2%] vs. 40 of 283 patients [14.1%]; odds ratio, 0.29; 95% CI, 0.16 to 0.53). The large differences between the findings of these studies and those of our study may be explained by several factors. First, the

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Subgroup	No. of Patients	Benznidazole	Placebo	Hazard Ratio (95% CI)	P Value for Interaction
		% of patients			
Country					0.16
Colombia or El Salvador	580	24.1	25.6		
Brazil	1358	33.2	37.6		
Argentina or Bolivia	916	21.4	18.5		
PCR status at baseline					0.96
Positive	1148	24.6	26.9		
Negative	748	23.7	25.3		
Age					0.56
≤56 yr	1428	22.8	25.6		
>56 yr	1426	32.1	32.7		
Sex					0.81
Female	1445	24.1	25.1		
Male	1409	30.8	33.4		
Disease-severity score					0.55
Low	1309	16.8	16.2		
Intermediate	890	29.9	33.3		
High	655	45.1	50.0		
Left ventricular ejection fraction					0.45
<40%	389	62.5	63.0		
≥40%	2465	21.8	23.9		
Left ventricular end diastolic diameter					0.41
<50 mm	674	13.7	17.9		
≥50 mm	1548	34.6	36.6		
Use of amiodarone					0.008
No	2302	24.7	24.1		
Yes	551	39.1	50.9		
Use of spironolactone					0.49
No	2375	21.6	23.5		
Yes	478	56.8	57.0		
Regional wall-motion abnormality					0.92
No	1397	21.2	22.4		
Yes	853	41.1	43.6		
Low QRS voltage					0.88
No	2342	28.7	29.9		
Yes	341	25.8	28.2		
RBBB and left anterior fascicular block					0.46
No	1946	28.6	29.2		
Yes	907	25.4	29.0		

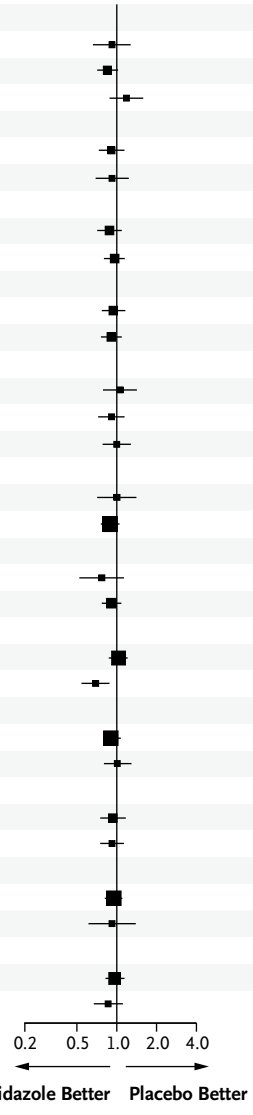

Figure 2. Primary Outcome, According to Subgroup.

Shown are hazard ratios for the primary outcome in all 12 prespecified subgroups in the benznidazole group and the placebo group, according to clinical characteristics and results on electrocardiography and two-dimensional echocardiography indicating the severity of disease. Rates of the primary outcome in the two groups are presented with a mean of 5.4 years of follow-up. The size of the squares is proportional to the size of the subgroup. The subgroup according to age was defined on the basis of the median age of 56 years. The disease-severity score was calculated as follows: New York Heart Association class III (5 points), cardiothoracic ratio of more than 0.5 (5 points), regional wall-motion abnormalities (3 points), complex ventricular arrhythmias (3 points), low-voltage QRS complex (2 points), and male sex (2 points). A low score ranges from 0 to 2, an intermediate score ranges from 3 to 5, and a high score is more than 5. For the primary outcome, the presence or absence of baseline therapy with amiodarone was the only subgroup that showed significant heterogeneity. PCR denotes polymerase chain reaction, and RBBB right bundle-branch block.

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Subgroup	No. of Patients	Benznidazole	Placebo	Odds Ratio (95% CI)	P Value for Interaction
		% of patients			
Country					<0.001
Colombia or El Salvador	317	43.9	45.6		
Brazil	213	86.3	24.3		
Argentina or Bolivia	388	73.0	28.6		
PCR status at baseline					
Positive	918	66.2	33.5		
Negative	596	70.3	59.4	***	
Age					0.31
≤56 yr	442	66.2	31.0		
>56 yr	476	66.2	35.8		
Sex					0.12
Female	447	62.4	33.6		
Male	471	69.3	33.5		
Disease-severity score					0.76
Low	333	73.1	31.8		
Intermediate	360	61.7	37.8		
High	225	63.0	29.1		
Left ventricular ejection fraction					0.52
<40%	110	70.0	30.0		
≥40%	808	65.7	34.0		
Left ventricular end diastolic diameter					0.85
<50 mm	237	62.2	30.2		
≥50 mm	457	65.3	36.1		
Use of amiodarone					0.33
No	802	64.7	33.9		
Yes	116	76.4	31.2		
Use of spironolactone					0.40
No	803	65.2	32.8		
Yes	115	73.6	38.7		
Regional wall-motion abnormality					0.62
No	441	66.3	35.2		
Yes	262	60.6	32.6		
Low QRS voltage					0.12
No	706	67.3	31.6		
Yes	153	63.4	41.5		
RBBB and left anterior fascicular block					0.14
No	671	63.7	31.6		
Yes	247	72.9	38.8		

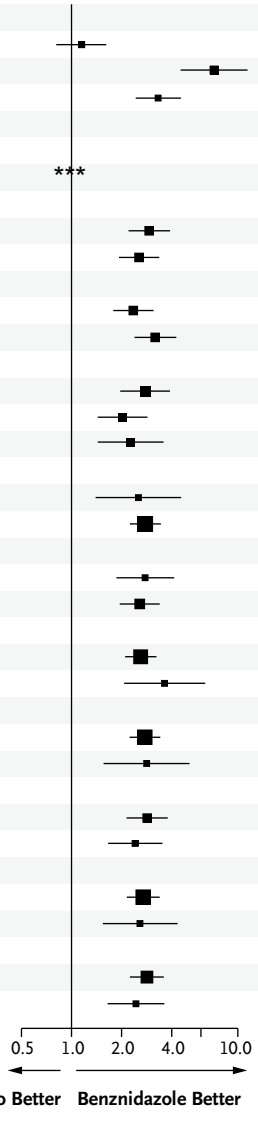

Figure 3. Conversion to Negative Results on PCR, According to Subgroup.

Shown are odds ratios for conversion to negative results for *Trypanosoma cruzi* on PCR assay at the end of the treatment period, according to 11 of the 12 prespecified subgroups. The size of the squares is proportional to the size of the subgroup. Odds ratios and 95% confidence intervals were calculated from repeated-measures analysis with the use of generalized estimating equations (GEE), with a comparison of values at all three follow-up periods (after treatment, at 2 years, and at 5 years or more) with those at baseline. (Odds ratios for patients' PCR status at baseline are not shown, as indicated by asterisks, because these data were not analyzed in a GEE model.)

previous studies were small and most were nonrandomized, and the studies enrolled primarily patients without cardiomyopathy. Second, the rates of loss to follow-up were higher than those in our study (20% vs. 0.5%). Third, the studies did not evaluate outcomes such as total rates of death, heart failure, or any of the composite outcomes that we analyzed.

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Table 3. Adverse Events and Laboratory Abnormalities.*

Cohort and Event	Adverse Events Leading to Drug Interruption			Serious Adverse Events Leading to Drug Interruption		
	Benznidazole (N = 1431)	Placebo (N = 1423)	P Value	Benznidazole (N = 1431)	Placebo (N = 1423)	P Value
	no./total no. (%)			*no./total no. (%)*		
Patients completing follow-up visits through end of study-treatment period	1429/1431 (99.9)	1422/1423 (99.9)		1429/1431 (99.9)	1422/1423 (99.9)	
Any adverse event	342/1429 (23.9)	135/1422 (9.5)	<0.001	119/1429 (8.3)	20/1422 (1.4)	<0.001
Cutaneous rash	137/1429 (9.6)	18/1422 (1.3)	<0.001	58/1429 (4.1)	2/1422 (0.1)	<0.001
Gastrointestinal symptoms	112/1429 (7.8)	41/1422 (2.9)	<0.001	26/1429 (1.8)	9/1422 (0.6)	0.004
Nervous system symptoms including peripheral neuropathy	52/1429 (3.6)	19/1422 (1.3)	<0.001	14/1429 (1.0)	6/1422 (0.4)	0.07
Leukopenia†	2/1429 (0.1)	2/1422 (0.1)	1.0	1/1429 (0.1)	0	NA
Permanent treatment discontinuation	192/1429 (13.4)	51/1422 (3.6)	<0.001	96/1429 (6.7)	15/1422 (1.1)	<0.001
Patients completing 60-day visit‡	1123/1431 (78.5)	1194/1423 (83.9)		0	0	NA
Alanine aminotransferase >2× ULN	55/1123 (4.9)	19/1194 (1.6)	<0.001	0	0	NA
Alanine aminotransferase >3× ULN	20/1123 (1.8)	9/1194 (0.8)	0.03	0	0	NA

* NA denotes not applicable, and ULN upper limit of the normal range.
† Leukopenia was defined as a neutrophil count of less than 1900 cells per cubic millimeter.
‡ Data are shown for patients who completed the 60-day study visit and had available values for alanine aminotransferase at that visit.

Benznidazole typically clears parasite detection in approximately 94% of patients who are in the indeterminate phase of the disease (i.e., have positive serologic findings but no evidence of cardiac damage), with effects sustained for at least 1 year.[34] In contrast, in our study involving only patients with established Chagas' cardiomyopathy, the efficacy of treatment as assessed by conversion to negative PCR results was more modest, with rates of 66% at the end of treatment and 46.7% after 5 years or more, which suggests that the standard regimen of benznidazole may be less effective in patients with established cardiomyopathy. Genetic subtypes of *T. cruzi* vary according to the geographic location. Since we did not have genotype information, we had to infer that patients in certain geographic regions were likely to have distinct *T. cruzi* strains. On the basis of this assumption, we observed significant differences among countries in the PCR response to therapy (Fig. 3). This finding may explain the lack of overall reduction in the primary outcome, but we did not observe consistently parallel results on clinical outcomes. We did not find any significant effect of therapy on the primary outcome even after adjustment for disease severity or the risk status of the patients. Only the subgroup receiving amiodarone (a high-risk group) appeared to benefit from therapy, but this result should be viewed with caution, since a large number of subgroup analyses were performed and rates of parasite detection on PCR in the benznidazole group were similar regardless of the receipt of amiodarone at baseline. However, this intriguing observation may require further exploration.[35]

Although our study was a large trial involving patients with established Chagas' cardiomyopathy, we could have missed small differences in risk (e.g., a relative risk reduction of 10%). The 95% confidence intervals in our analyses rule out a relative risk reduction of 20%, which is smaller than the difference that the study was designed to detect. Variable responses to benznidazole have been reported previously and may have contributed to our neutral findings.[36] Future analyses of stored blood samples by means of quantitative PCR and genotyping may provide a more precise characterization of *T. cruzi* that can be used to assess whether genotype influences the clinical response to benznidazole.

It is possible that the benefit of benznidazole

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may be observed in patients who are at very low risk before the appearance of cardiac damage or that the benefit may accrue with more prolonged therapy (as is the case with therapy for some other chronic infections, such as tuberculosis or leprosy), with repeated pulses of benznidazole, or with treatment at an earlier stage of the disease. These hypotheses are untested. Whether longer follow-up is needed to detect the emergence of a benefit is also a consideration but is speculative, since 60% of our patients were followed for more than 6 years and 25% for more than 7 years, and no obvious signal of possible benefit was observed.

Our findings do not challenge current guidelines that recommend treatment with trypanocidal therapy in the early stages of chronic Chagas' infection (which are based on several studies,[37,38] including one that showed the benefit in preventing congenital transmission[39]) and should not detract from the pursuit of general goals for exploring more effective or earlier treatments with new drugs or drug combinations.[40] It is notable that 13.4% of patients permanently discontinued treatment with benznidazole because of adverse events, a rate that is lower than that previously reported in observational and small, randomized trials and supports the concept that repeated trypanocidal treatment may be feasible.

In conclusion, among patients with established Chagas' cardiomyopathy, benznidazole treatment significantly reduced the detection of circulating parasites but did not reduce cardiac clinical progression.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

APPENDIX

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REFERENCES

1. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Wkly Epidemiol Rec 2015;90:33-43.
2. Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. Lancet 2010;375:1388-402.
3. Bern C. Chagas' Disease. N Engl J Med 2015;373:456-66.
4. Organización Panamericana de la Salud. Estimación cuantitativa de la enfermedad de Chagas en las Américas. Montevideo, Uruguay: Organización Panamericana de la Salud, 2006:1-28. (Publication no. OPS/HDM/CD/425-06.)
5. World Health Organization. Annex table 3: burden of disease in DALYs by cause, sex, and mortality stratum in WHO regions, estimates for 2002. World Health Rep 2002 (http://www.who.int/whr/2002/whr2002_annex3.pdf).
6. Rassi A, Rassi A Jr, Rassi GG. Fase aguda. In: Brener Z, Andrade ZA, Barral Neto M. *Trypanosoma cruzi* e doença de Chagas. 2nd ed. Rio de Janeiro: Guanabara Koogan Editora, 2000:231-45.
7. Marin-Neto JA, Rassi A Jr, Maciel BC, Simoes MV, Schmidt A. Chagas heart disease. In: Yusuf S, Cairns JA, Camm AJ, Fallen EL, Gersh BJ, eds. Evidence-based cardiology. 3rd ed. London: BMJ Books, 2010:823-41.
8. Rassi A Jr, Rassi A, Little WC, et al. Development and validation of a risk score for predicting death in Chagas' heart disease. N Engl J Med 2006;355:799-808.
9. Marin-Neto JA, Cunha-Neto E, Maciel BC, Simões MV. Pathogenesis of chronic Chagas heart disease. Circulation 2007;115:1109-23.
10. Marin-Neto JA, Rassi A Jr, Maciel BC. Pathology and pathogenesis of Chagas disease. In: McKenna WJ, Weller PF, Yeon SB, Baron EL, eds. UpToDate in cardiovascular medicine, a CD-ROM textbook. version 5.11. Wellesley, MA: UpToDate in Medicine, 2015.

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11. Cunha-Neto E, Coelho V, Guilherme L, Fiorelli A, Stolf N, Kalil J. Autoimmunity in Chagas' disease: identification of cardiac myosin-B13 Trypanosoma cruzi protein crossreactive T cell clones in heart lesions of a chronic Chagas' cardiomyopathy patient. J Clin Invest 1996;98:1709-12.
12. Iwai LK, Juliano MA, Juliano L, Kalil J, Cunha-Neto E. T-cell molecular mimicry in Chagas disease: identification and partial structural analysis of multiple cross-reactive epitopes between Trypanosoma cruzi B13 and cardiac myosin heavy chain. J Autoimmun 2005;24:111-7.
13. Jones EM, Colley DG, Tostes S, Lopes ER, Vnencak-Jones CL, McCurley TL. A Trypanosoma cruzi DNA sequence amplified from inflammatory lesions in human chagasic cardiomyopathy. Trans Assoc Am Physicians 1992;105:182-9.
14. Higuchi MdeL, De Brito T, Martins Reis M, et al. Correlation between Trypanosoma cruzi parasitism and myocardial inflammatory infiltrate in human chronic chagasic myocarditis: light microscopy and immunohistochemical findings. Cardiovasc Pathol 1993;2:101-6.
15. Bellotti G, Bocchi EA, de Moraes AV, et al. In vivo detection of Trypanosoma cruzi antigens in hearts of patients with chronic Chagas' heart disease. Am Heart J 1996;131:301-7.
16. Andrade SG, Stocker-Guerret S, Pimentel AS, Grimaud JA. Reversibility of cardiac fibrosis in mice chronically infected with Trypanosoma cruzi, under specific chemotherapy. Mem Inst Oswaldo Cruz 1991;86:187-200.
17. Garcia S, Ramos CO, Senra JFV, et al. Treatment with benznidazole during the chronic phase of experimental Chagas' disease decreases cardiac alterations. Antimicrob Agents Chemother 2005;49:1521-8.
18. Coura JR, de Abreu LL, Willcox HP, Petana W. Comparative controlled study on the use of benznidazole, nifurtimox and placebo, in the chronic form of Chagas' disease, in a field area with interrupted transmission. I. Preliminary evaluation. Rev Soc Bras Med Trop 1997;30:139-44. (In Portugese.)
19. Villar JC, Marin-Neto JA, Ebrahim S, Yusuf S. Trypanocidal drugs for chronic asymptomatic Trypanosoma cruzi infection. Cochrane Database Syst Rev 2002;1:CD003463.
20. Viotti R, Vigliano C, Lococo B, 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.
21. Fabbro DL, Streiger ML, Arias ED, Bizai ML, del Barco M, Amicone NA. Trypanocide treatment among adults with chronic Chagas disease living in Santa Fe City (Argentina), over a mean follow-up of 21 years: parasitological, serological and clinical evolution. Rev Soc Bras Med Trop 2007;40:1-10.
22. Villar JC, Perez JG, Cortes OL, et al. Trypanocidal drugs for chronic asymptomatic *Trypanosoma cruzi* infection. Cochrane Database Syst Rev 2014;5:CD003463
23. Marin-Neto JA, Rassi A Jr, Morillo CA, et al. 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.
24. Schijman AG, Bisio M, Orellana L, et al. International study to evaluate PCR methods for detection of Trypanosoma cruzi DNA in blood samples from Chagas disease patients. PLoS Negl Trop Dis 2011;5(1):e931.
25. Wincker P, Britto C, Pereira JB, Cardoso MA, Oelemann W, Morel CM. Use of a simplified polymerase chain reaction procedure to detect Trypanosoma cruzi in blood samples from chronic chagasic patients in a rural endemic area. Am J Trop Med Hyg 1994;51:771-7.
26. Ramírez JD, Guhl F, Rendón LM, Rosas F, Marin-Neto JA, Morillo CA. Chagas cardiomyopathy manifestations and Trypanosoma cruzi genotypes circulating in chronic Chagasic patients. PLoS Negl Trop Dis 2010;4(11):e899.
27. Zingales B, Miles MA, Campbell DA, et al. The revised Trypanosoma cruzi subspecific nomenclature: rationale, epidemiological relevance and research applications. Infect Genet Evol 2012;12:240-53.
28. Zingales B, Miles MA, Moraes CB, et al. Drug discovery for Chagas disease should consider *Trypanosoma cruzi* strain diversity. Mem Inst Oswaldo Cruz 2014;109:828-33.
29. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 1986;42:121-30.
30. de Andrade AL, Zicker F, de Oliveira RM, et al. Randomised trial of efficacy of benznidazole in treatment of early Trypanosoma cruzi infection. Lancet 1996;348:1407-13.
31. Sosa Estani S, Segura EL, Ruiz AM, Velazquez E, Porcel BM, Yampotis C. Efficacy of chemotherapy with benznidazole in children in the indeterminate phase of Chagas' disease. Am J Trop Med Hyg 1998;59:526-9.
32. Pérez-Molina JA, Pérez-Ayala A, Moreno S, Fernández-González MC, Zamora J, López-Velez R. Use of benznidazole to treat chronic Chagas' disease: a systematic review with a meta-analysis. J Antimicrob Chemother 2009;64:1139-47.
33. Kuschnir E, Sgammini H, Castro R, Evequoz C, Ledesma R, Brunetto J. Evaluation of cardiac function by radioisotopic angiography, in patients with chronic Chagas cardiopathy. Arq Bras Cardiol 1985;45:249-56. (In Spanish.)
34. Molina I, Gómez i Prat J, Salvador F, et al. Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. N Engl J Med 2014;370:1899-908.
35. Carmo AA, Rocha MO, Silva JL, et al. Amiodarone and Trypanosoma cruzi parasitemia in patients with Chagas disease. Int J Cardiol 2015;189:182-4.
36. Veloso VM, Carneiro CM, Toledo MJO, et al. Variation in susceptibility to benznidazole in isolates derived from Trypanosoma cruzi parental strains. Mem Inst Oswaldo Cruz 2001;96:1005-11.
37. Bern C, Montgomery SP, Herwaldt BL, et al. Evaluation and treatment of chagas disease in the United States: a systematic review. JAMA 2007;298:2171-81.
38. Andrade JP, Marin Neto JA, Paola AA, et al. I Latin American guidelines for the diagnosis and treatment of Chagas' heart disease: executive summary. Arq Bras Cardiol 2011;96:434-42.
39. Fabbro DL, Danesi E, Olivera V, et al. Trypanocide treatment of women infected with Trypanosoma cruzi and its effect on preventing congenital Chagas. PLoS Negl Trop Dis 2014;8(11):e3312.
40. Tarleton RL, Gürtler RE, Urbina JA, Ramsey J, Viotti R. Chagas disease and the London declaration on neglected tropical diseases. PLoS Negl Trop Dis 2014;8(10):e3219.

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