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Amiodarone and *Trypanosoma cruzi* parasitemia in patients with Chagas disease

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Chagas disease (ChD) is a main cause of cardiopathy in Latin America, causing thousands of deaths each year.(1) Amiodarone has been widely used as antiarrhythmic drug to prevent recurrences of malignant ventricular arrhythmias in patients with ChD. Although the antiarrhythmic effects of amiodarone are well known, there are some evidences that it also has anti-*Trypanosoma cruzi* activity,(2) disrupting Ca^{2+} homeostasis and blocking oxidosqualenecyclase enzyme in *T. cruzi*, causing ultrastructural damage.(3) Nevertheless, data showing antiparasitic effects of amiodarone in human are lacking, with only one case report demonstrating decrease in parasite load after amiodarone use.(4)

We evaluated whether patients in use of amiodarone have lower parasite load than matched infected subjects, employing a recently developed target-capture real-time PCR assay that can detect as few as one parasite per 20mL of processed blood(5), in a previously described cohort of Chagas disease subjects studied by the National Heart, Lung and Blood Institute (NHLBI) Retrovirus Epidemiological Donor Study-II (REDS-II).(6-8)

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The REDS-II cohort of ChD subjects included 499 *T. cruzi* seropositive subjects recruited among blood donors and 101 patients previously diagnosed as Chagas cardiomyopathy from the Heart Institute of the University of Sao Paulo. From 2008 to 2010, recruited individuals were submitted to a questionnaire and medical evaluation, resting 12-lead, echocardiogram and blood draw for NT-proBNP and PCR measurements.(7, 8) Soronegative donors used as the control group in the original study were excluded from this analysis.

Resting 12-lead ECGs were recorded using General Electric MAC 1200 electrocardiograph (GE Healthcare, Waukesha, WI). ECGs were analyzed electronically and classified by Minnesota code criteria by a central ECG laboratory (EPICARE, Wake Forest University, Winston-Salem, NC). NT-proBNP levels in plasma were measured using VITROS System (Ortho Clinical Diagnostics, Raritan, NJ, US).(7)

Echocardiograms were performed using a Sequoia 512 ultrasound instrument (Acuson, Mountain View, CA, USA) or a GE Vivid3 (GE Healthcare, Waukesha, WI). Cardiac measurements were performed according to the guidelines of the American Society of Echocardiography. All measurements were analyzed offline at the Cardiovascular Branch, Echocardiography Laboratory, NHLBI/NIH, Bethesda, Maryland, US.(6)

Chagas cardiomyopathy was recognized by the presence of a predefined set of abnormalities in clinical examination, echocardiogram or ECG measurements, according to the evaluation of an expert panel, as detailed elsewhere.(6)

Twenty milliliters of whole blood were collected for quantitative, real-time PCR. Aliquots were frozen in Brazil at -20°C until shipped to the US REDS-II Central Laboratory on dry ice, followed by maintenance at -70°C. Subsequently, PCR assays were performed by amplification of kinetoplastminicircle DNA sequences of *T. cruzi*.(9)

We recognized ChD subjects in use of amiodarone among the whole cohort and used a new matching method, Genetic Matching (GenMatch), to select a comparison group of ChD patients with similar characteristics in order to allow the evaluation of the effect of amiodarone on parasitemia presence and intensity. GenMatch is a method of multivariate matching that uses an evolutionary search algorithm to determine the weight each covariate is given, in order to maximize the balance in the distribution of baseline characteristics between both groups.(10) In the entire cohort, there were 37 patients using amiodarone. These patients were matched in a 1:1 ratio by age, gender, years of exposure to Chagas disease, New York Heart Association (NYHA) Functional class, definite cardiomyopathy, use of β -blockers, angiotensin converter enzyme (ACE) inhibitors or angiotensin-receptorblockers, QRS duration, major and minor ECG abnormalities, left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVED), segmental wall-motion abnormality, left ventricular lateral and septal E/e' ratio, and NT_proBNP levels (table 1). Balance was measured with standardized mean difference; a difference of 10% was taken to indicate meaningful imbalance. All the statistical analyses were conducted using R (Version 3.0.1).

This study protocol follows the 1975 Declaration of Helsinki and was approved by the Brazilian National Ethics Committee (CONEP# 1312/2006). Written informed consent was obtained from all subjects.

The mean daily dose of amiodarone was $205\text{mg} \pm 54\text{mg}$. In unmatched analysis, patients on amiodarone treatment used more frequently β -blocker and ACE inhibitors or angiotensin-receptor blockers and showed more frequently left ventricular dysfunction and dilatation in the echocardiographic study, as well ECG abnormalities. After GenMatch, the baseline characteristics became similar in both groups, except for gender and NT_proBNP levels (table 1).

The qualitative analysis of PCR showed positive results in 86% patients on amiodarone treatment and in 58% control patients. After matching, PCR was positive in 69% of paired control subjects ($p = 0.13$), demonstrating no difference between both groups.

T. cruzi DNA concentrations in blood were higher in patients on amiodarone treatment (1.3 parasite/20 mL vs 0.7 parasite/20 mL), but after GenMatch, quantitative analysis yielded similar results (1.3 parasites/20 mL vs 1.0 parasites/20 mL; $p=0.63$), also showing no statistical difference between patients treated or not with amiodarone.

Ventricular arrhythmia is a frequent and ominous finding in ChD patients(1) and the presence of parasitemia in chronic infected patients is related to the disease severity and may have prognostic importance.(9) Thus, a double effect of amiodarone, both as antiarrhythmic and anti-parasite drug, would be highly desirable and could affect favorably the natural history of Chagas cardiomyopathy. Although there are some experimental evidences of this anti-*T. cruzi* activity of amiodarone, our cohort did not show difference nor in qualitative PCR, neither in parasite load in patients using this drug, even after balancing baseline differences between groups in potential confounding factors.

This study has several limitations. First, this study was a retrospective analysis of data from REDS-II study and it was not designed for this purpose. Second, matching techniques were necessary to balance both populations and even in well balanced populations hidden bias can be present. Third, the number of patients using amiodarone is relatively small and there was limited information about duration of therapy.

In conclusion, using a well validated PCR assay to measure parasitic load in blood stream of ChD patients, we could not demonstrate lower levels of *T. cruzi* parasitemia in habitual users of amiodarone, as compared to a matched sample of ChD subjects.

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Table 1

Patients characteristics before and after Genetic Matching

	Unmatched			Genetic Matching		
	Amiodarone users N= 37	Non-amiodarone users N = 564	Std mean diff (%)	Amiodarone users N= 37	Non-amiodarone users N = 37	Std mean diff (%)
General features						
Age - yrs	49.7	48.2	4.1	49.7	49.7	0.0
Gender - % male	33.3	47.3	-33.0	33.3	27.1	16.1
Years of exposure	17.6	17.7	-0.6	17.6	17.4	1.7
NYHA Functional Class - % in class II	16.3	6.4	45.7	16.3	18.4	-6.4
Cardiomyopathy - %	91.7	40.1	70.2	91.7	91.3	0.6
Beta-blockers - %	72.2	16.8	96.3	72.2	72.4	-0.3
ACE inhibitors or angiotensin-receptor blockers - %	80.6	24.5	86.9	80.6	78.0	3.9
Electrocardiogram						
QRS duration - ms	138.6	102.1	35.8	138.6	134.5	3.1
Major ECG abnormalities - %	16.7	4.8	62.0	16.7	14.4	8.7
Minor ECG abnormalities - %	5.6	4.3	8.8	5.6	6.4	-3.7
Echocardiogram						
Left ventricular ejection fraction - %	31.8	56.7	-102.6	31.8	32.3	-2.0
Left ventricular end-diastolic diameter - mm	63.1	47.9	33.7	63.1	61.8	2.9
Segmental wall-motion abnormality - %	85.8	21.1	96.3	85.8	85.9	-0.1
Left ventricular septal E/e' ratio	11.8	7.9	43.9	11.8	12.4	-6.4
Left ventricular lateral E/e' ratio	9.7	6.0	51.5	9.7	9.1	8.5
Natriuretic peptides						
NT_proBNP (pg/mL)	2191.9	371.5	86.1	2191.9	1888.3	13.7

Data expressed in mean values or proportions.