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Chagas Myocarditis: a Convenient Model to Validate ECG Pathological Parameters in NMRI mice

Giraldo Elidiosmar[1], Santeliz Sonia[2], Yustiz María-Daniela[1], Rodriguez Hector[1,3] and Bonfante-Cabarcas Rafael[1]*

[1]*Unidad de Bioquímica, Decanato de Ciencias de la Salud*

[2]*Unidad de Biomedicina, Departamento de Medicina y Cirugía, Decanato de Ciencias Veterinarias; Universidad Centroccidental "Lisandro Alvarado". Barquisimeto, Lara, Venezuela*

[3]*Instituto Venezolano de Investigaciones Científicas (IVIC) - Centro de Biofísica e Bioquímica, Caracas, Venezuela*

***Corresponding Author:** Rafael Bonfante Cabarcas, Unidad de Bioquímica, Decanato de Ciencias de la Salud, Av. Libertador con Andrés Bello, Universidad Centroccidental "Lisandro Alvarado" Barquisimeto, Estado Lara, Venezuela.

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Abstract

The extensive advance in the development of transgenic mice technology in the cardiac pathology field requires an accurate knowledge of pathological electrocardiographic traces in murine models that must be clearly different from traces obtained in healthy mice and also reproduce electrocardiographic patterns observed in human cardiac pathologies. Chagas' disease could be an excellent model to distinguish between normal and abnormal murine electrocardiographic patterns, since chagasic myocarditis has a well-defined and widely characterized electrocardiographic signs. In order to identify abnormal electrocardiographic signs in NMRI mice, contrast electrocardiographic analysis between healthy and chagasic was accomplished. Electrocardiographic records were done under general anesthesia on 74 healthy and 174 chagasic mice. Results showed that the electrocardiographic profiles associated to mice with chagasic myocarditis were: atrial disturbances represented as negative, bimodal or double dissimilar P wave; myocarditis revealed a decrease in the amplitude of the QRS complex (mainly R wave) and lengthening of PR, QRS and QT intervals; ventricular repolarization disorders visualized as a decrease in the S wave rise, flattening of J wave which also it can delineate a plateau instead of a peak, prolonged J wave decay, TP segment above the isoelectric line, J wave inversion and U waves emerging from J wave decay that could represent early after depolarizations. In conclusion, the electrocardiographic data associated with mice with acute chagasic myocarditis reflect pathological electrocardiographic characteristics that could be used in the electrocardiographic analysis in cardiac disease mice models.

Keywords: *ECG, electrocardiography; NMRI mice; Chagas myocarditis; atrial disturbances; repolarization disturbances; early after depolarization*

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Introduction

The heart is an electrogenic organ that during the cardiac cycles generates defined electrical phenomena, which can be registered and analyzed by electrocardiography. The data generated by the electrocardiogram (ECG) is used to characterize and define both physiological and pathological patterns in living individuals, allowing adequate patient with cardiac diseases follow up, both in humans and in murine models. The characteristics of the human electrocardiogram were first described in 1895 by Einthoven [1], while the first detailed description of the mouse ECG was reported by Golbarg., *et al.* in 1968 [2].

The interest in mouse electrophysiology is expanding fast, mainly because the mouse transgenic technology is being used as a starting point for studying cardiovascular diseases, especially those with electrogenic disorders, such as channelopathies [3]. However, the usefulness of electrocardiographic studies need to be addressed in two aspects: first the extent of functional and pathological similarities between electrocardiographic traces recorded in murine models compared to their human counterpart, for example, mouse ECG is distinctively different from human, in that it is difficult to discern the onset and termination of a repolarization [4]. Secondly, the real electrogenic significance of the waves recorded in the murine models, in healthy situations as well as in mice with cardiac disease, due to the electrocardiographic variability observed between the different strains of mice and the theoretical and practical background of the researchers who work with murine models [4-6]. Despite substantial differences in the morphology of the ECG in mice and man, similar criteria are used for analysis.

The murine ECG has some unusual characteristics that make interpretation of the phenotypic expression of the changes in the ventricular repolarization uncertain. The QRS wave complex is followed by a secondary slower deflection which has been called J or T wave, and successively by a sometimes subtle negative T wave. In consequence it has been suggested that, depolarization in some regions of the murine heart occur simultaneously with repolarization in other areas; and the QRS in the mouse, corresponds not to a depolarization spreading but to both the spreading of the depolarization and an early repolarization across the myocardium [4,7-9].

Chagasic cardiomyopathy could represents an excellent model to determine the degree of similarity between the characteristics of human and murine electrocardiograms, as well as to better characterize the meaning of the waves in the murine electrocardiogram.

There are crucial data supporting the claim that Chagas disease is an unresolved global public health problem. In Latin America endemic countries, the residual prevalence is a distinctive phenomenon of about 7.56%, an incidence of 0.39 and a mortality of 0.16 cases per thousand inhabitants [10,11]. Moreover, epidemic outbreaks with high morbidity and mortality in urban areas [12], suggest a more serious underlying problem where accidental food contamination could have been the product of a high density sources of infection. In consequence, Chagas disease is considered the vector transmitted parasitic disease with the greatest socioeconomic impact in Latin America, responsible for the loss of productivity at an estimated cost of US$ 1.2 billion annually, with a significant impact on Latin American countries income per capita [13]. Furthermore, the migration of seropositive individuals from endemic countries to developed countries, seeking socioeconomic improvements, has led to the export of prevalence; a 4.2% migrant individuals living in these countries has been reported as infected, increasing the risk of transmission by blood transfusion and by transplacental pathway [14].

Chagasic cardiomyopathy is characterized by multiple electrocardiographic disorders such as conduction disorders, rhythm disorders, cavity enlargement and disorders in the ventricular repolarization [15]. All of these disorders reflect the damage of myocardial cells and conduction tissues caused by the parasite *Trypanosoma cruzi*, by cardiac inflammation generated by the host's immune response with subsequent cardiac remodeling and by ischemia caused by alterations in the endothelium and formation of platelet and hematic thrombi in the coronary microvasculature [16,17].

Hallmark electrocardiographic disorders of Chagas' disease have been widely characterized in humans; however, since the murine model is the preferred choice for experimental studies of Chagas' disease, there are no detailed studies that have yet accurately characterized electrocardiographic disorders in chagasic mice. In this regard, our group has been able to partially characterize the

electrocardiographic characteristics in mice with chagasic cardiomyopathy, as well as the effect of trypanocidal treatments in the reversibility of ECG disorders [18,19].

Because mice are the preferred host for developing transgenic cardiac disease models, it is imperative to identify and characterize the electrocardiographic disorders associated with heart electrogenic dysfunction and to clearly differentiate them from the morphological normal variants observed in healthy mice with preserved cardiac function, with the purpose of delimitating and orienting a particular electro pathological diagnosis.

Because of this, in the present paper we present a detailed comparative characterization of the electrocardiogram in both healthy NMRI mice and those with acute chagasic cardiomyopathy; with the purpose of delineating the electrocardiographic wave morphologies that have pathological significance, in order to elucidate and discern the physiological and pathological meaning of each electrocardiographic waves, and also to showcase the most frequent electrocardiographic disorders in acute chagasic cardiomyopathy in NMRI mice.

Materials and Methods

Sample: The sample consisted of 248 NMRI adult male mice, between 2 and 4 months old, weighing between 30-50 gr, obtained from animal research facilities at the Universidad Centroccidental "Lisandro Alvarado" University (Barquisimeto, Venezuela); these mice belong to the data base of our laboratory gathered from different studies. The mice were distributed in stainless steel cages with 29x30x14 cm dimensions, in a number of 5 to 8 individuals per cage, with free access to water and animal food in pellets (Protinal, Venezuela), with 12 hours light/dark cycles and an average 27°C temperature.

The Chagas myocardiopathy group consisted of 174 mice infected intraperitoneally with blood trypomastigotes suspended 0.9% NaCl, at a dose of 100 trypomastigotes per gram of body weight. The strain of TCI lineage used has been registered in the WHO bank under the name MHOM/VEcepa792/2-92-YBM and fully characterized [18,19]. Parasites assayed were obtained from the blood of infected mice in the acute phase of Chagas disease and maintained in vector/mouse cycles; vectors were Rhodnius prolixus stage III nymphs. In this model the parasitemia increased considerably at the third week reaching peak values between 5 to 20 x 10^6 parasites/ml. Mortality during the sixth week ranged from 80% to 90% of individuals, most of the mice died of congestive heart failure.

Bioethics: All experimental procedures performed in this study were based on the principles established in the bioethics and biosafety manual of the National Fund for Science and Technology, Ministry of Popular Power for Science and Technology, Venezuela.

Electrocardiography: Electrocardiographic studies were performed, under anesthesia with sodium pentobarbital and ketamine, 25-50 mg/kg body weight each via ip, in bipolar configuration using 3 needle-type electrodes (one positive, one negative and one neutral or reference), positioned in the subcutaneous tissue. We worked with 4 lead-ECG configurations: the DI positive electrode was placed together with the reference electrode on the left shoulder joint, while the negative electrode was positioned on the right shoulder joint; the DII positive electrode was positioned on the xiphoid process, the negative one was located in the right shoulder joint and the reference one on the left shoulder joint; in DIII both the negative electrode and reference electrode were placed on the left shoulder joint and the positive electrode was maintained on the xiphoid process and the AVF lead negative electrode was positioned on the cervical midline above the suprasternal fossa, while the positive electrode was placed on the xiphoid process and the reference one remained on the left shoulder joint.

Electrodes were connected to a BioAmp amplifier (ADInstruments, New Zealand), analog signals were converted to digital signals through a Powerlab/8sp interphase system (ADInstruments, New Zealand), displayed, recorded and analyzed on a personal computer using the Chart v4.2.1 software (ADInstruments New Zealand). The signal capture was performed at a 1000 events/sec frequency and filtered at 60 Hz.

Histopathology: Heart samples were fixed with 10% formalin in PBS pH 7.4, embedded in paraffin, cut into microtome and stained with hematoxylin-eosin.

Data analysis: Data is expressed in absolute values, percentages or mean ± SEM. Unpaired Student's t test were used to analyze significance of observed differences between healthy and chagasic mice. To determine the association between a specific electrocardiographic morphology with healthy or chagasic mice, contingency tables were constructed and analyzed by the Fisher´s exact test. A $p < 0.05$ was accepted as significant. Graph Pad Prism Software 5.0 was used for statistical analysis.

Results

In Table I electrocardiographic parameters obtained from healthy and infected NMRI mice are presented. These parameters are the mean ± standard error (SEM) and the percentiles 10 and 90. When the means of each parameters where statistically compared between healthy and chagasic mice, we found that chagasic mice exhibited electrocardiographic disorders in virtually all parameters studied, except for the heart rate, S wave amplitude, P and QRS wave axis. From this table we can also predict pathological values; if the parameter value are increased in chagasic mice, the pathological values would be above the 90 percentile of healthy mice; on the contrary if the value are significantly decreased in chagasic mice, the pathological values would be below the 10 percentile of healthy mice.

In healthy animals the P wave presented a predominantly positive morphology in leads II and AVF, being monophasic or biphasic positive/negative, whereas in the II derivation a negative monophasic morphology predominated, being that these patterns were associated to healthy mice (Figure 1 and Table II). In these mice, a P amplitude of 105.7 ± 4.4 μV was obtained while the P axis was directed to the lower left quadrant at +47.7 ± 3.1° [Table I]

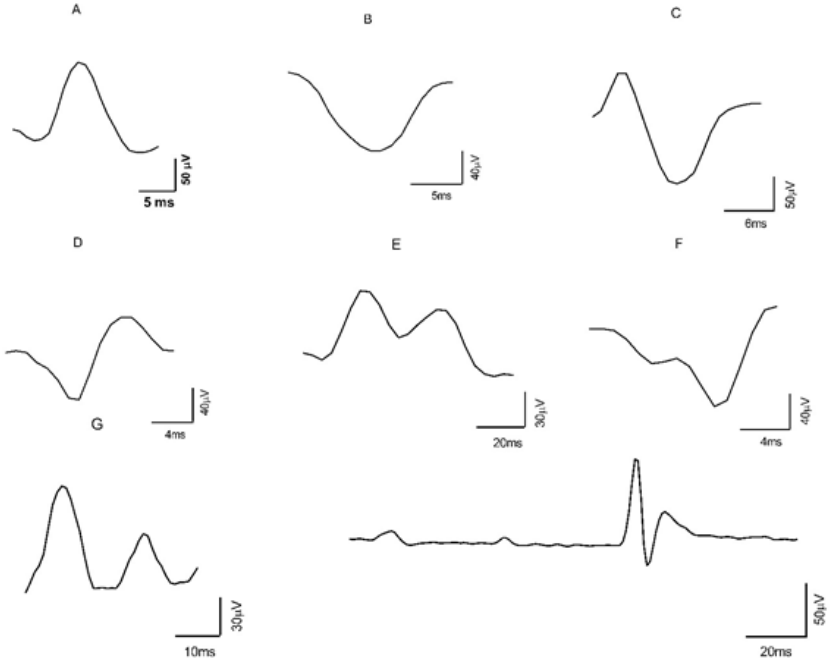

Figure 1: *Atrial disturbances. Panel A shows a normal monophasic positive p wave; panel B shows a negative p wave; panels C and D show a biphasic, either positive-negative or negative-positive p wave, respectively; panels E and F show bimodal p wave, either positive or negative; panel G and trace at bottom right corner show double non-similar p wave. Referential values of vertical and horizontal bars are indicated for each trace.*

Ecg Parameters	Healthy Mice			Chagasic Mice			P Value
	Mean ± SEM	Percentiles		Mean ± SEM	Percentiles		
		10%	90%		10%	90%	
HR	371.2 ± 5.8	250.2	498.9	380.1 ± 6.1	281.0	483.3	0,3067
PR	40.0 ± 0.4	34.1	46.9	↑49.2 ± 0.95*	35.3	62.6	< 0.0001
PR Bazett	96.4 ± 0.8	83.3	110.0	↑120.3 ± 2.00*	87.0	152.6	< 0.0001
QRS	10.6 ± 0.1	8.7	12.8	↑13.0 ± 0.23*	10.0	17.0	< 0.0001
QRS Bazett	25.2 ± 0.3	20.0	29. 0	↑31.7 ± 0.52*	24.0	41.0	< 0.0001
QT	41.6 ± 2.2	21.2	92.2	↑87.6 ± 2.6*	36.9	129.6	< 0.0001
QTc	97.3 ± 4.4	54.1	203.7	↑ 213.0 ± 5.3*	93.9	285.5	< 0.0001
Pa	105.7 ± 4.4	48.4	189.7	↓40.7 ± 4.6*	-28.5	101.3	< 0.0001
Ra	1348.0 ± 30.8	832.5	2073.0	↓728.6 ± 23.3*	332.4	1079.0	< 0.0001
Sa	-362.9 ± 19.8	-779.9	-20.4	-303.2 ± 23.4	-633.8	-8.3	0.0581
Ja	438.5 ± 10.4	241.7	654.8	↓211.3 ± 13.5*	-14.9	434.4	< 0.0001
Pa/Ra	8.1 ± 0.3	3.6	13.6	↓ 5.0 ± 1*	-4.8	13.9	0.0008
Sa/Ra	16.9 ± 2.2	-65.9	22.6	↓ 48.8 ± 4.5*	-111.0	-1.2	< 0.0001
Ja/aR	34.6 ± 0.9	19.5	53.9	↓29.6 ± 2.6*	-4.4	61.6	0.0377
P axis	47.7 ± 3,1	8.0	80.5	54.3 ± 4.3	15.1	94.6	0.3062
QRS axis	83.1 ± 1.1	75.0	89.0	71.3 ± 3.9	20.9	95.6	0.022
J axis	85.9 ± 0.4	81.0	89.0	↓ 58.8 ± 5.3*	77.2	94.0	< 0.0001
τ_1 of J decay	4.1 ± 0.1	2.9	5.0	↑7.7 ± 0.4*	3.6	14.3	< 0.0001
τ_1 %	90.5 ± 1.3	75.9	100.0	↓ 55.3 ± 2.8*	8.3	100.0	< 0.0001
τ_2 of J decay	67.3 ± 5.1	38.4	113.3	↓60.3 ± 4.4*	13.4	124.8	0.3662
τ_2 %	9.5 ± 1.1	0.0	24.2	↑48.1 ± 2.8*	8.6	91.1	< 0.0001
HR: Heart rate in beats/min; PR, QRS and QT segments lengths are expressed in ms; Pa, Ra, Sa and Ja amplitudes are expressed in μV; P, QRS and T axis are expressed in grades; τ_1 and τ_2 decays are expresed in ms. * means $p < 0.05$ as compared with healthy mice obtained by studen t test. ↓or ↑means whether parameters significantly decrease or increase.												

Table I: *Quantitative Electocardiographic Parameters in Healthy and Chagasic Mice.*

P Wave Morphologies	Healthy Mice						Chagasic Mice					
	DII		DIII		AVF		DII		DIII		AVF	
	n	%	n	%	n	%	n	%	n	%	n	%
Positive monophasic	59	79.7*	12	16.4	29	39.1	63	48.8	30	17.4	46	38.6
Negative monophasic	1	1.4	33	45.2*	11	14.9	3	2.3	45	26.2	11	9.2
Positive/negative byphasic	9	12.1	24	32.9*	28	37.8*	6	4.7	35	20.4	10	8.4
Negative/positive byphasic	2	2.7	1	1.4	1	1.4	3	2.3	4	2.3	3	2.6
Positive bimodal	1	1.4	0	0	1	1.4	18	13.9*	9	5.2	9	7.6
Negative bimodal	2	2.7	3	4.1	4	5.4	5	3.9	13	7.6	9	7.6
Double positive	0	0	0	0	0	0	26	20.2*	28	16.3*	25	21*
Doble positive/negative	0	0	0	0	0	0	0	0	1	0.6	1	0.8

No P	0	0	0	0	0	0	5	3.9	7	4	5	4.2
TOTAL	74	100	73	100	74	100	129	100	172	100	119	100
*means p < 0.05 when healthy mice and chagasic mice were compared using Fisher's exact test.												

Table II: *P Wave Morphologies in Healthy and Chagasic Mice.*

In the chagasic animals the P wave presented a similar morphological pattern to the healthy ones but with a significantly less frequency in individuals. In the chagasic mice, the patterns that were more frequently observed were: bimodal P waves, the synchronous presence of 2 individual P waves or multiple P waves or an absence of P waves; all of these characteristics had a statistically significant association with chagasic mice, because in healthy mice practically no such morphological pattern was observed. (Figure 1 and Table II). In the chagasic mice a significantly lower P wave amplitude was obtained with values of 40.7 ± 4.6 μV and a similar axis to the healthy mice at +54.3 ± 4.3° (Table I).

The PR interval was significantly prolonged in the chagasic mice with values of 49.15 ± 0.95 ms, compared to healthy mice that presented values of 40.02 ± 0.39 ms.

The QRS complex was significantly prolonged in chagasic mice with values of 13.04 ± 0.23 ms, compared to healthy mice that presented values of 10.63 ± 0.11 ms. We also observed that the QT interval was significantly prolonged in chagasic mice (Table I).

The Q wave could only be defined in few cases of each group, while the R wave was the most constant, always being a positive deflection, which was significantly higher in healthy mice with values of 1348.0 ± 30.8 μV, when compared to the chagasic mice that presented values of 728.6 ± 23.3 μV.

The S wave's absolute amplitude was higher in healthy mice with values of -362.9 ± 19.8 μV compared to the values of chagasic mice (-303.2 ± 23.4 μV), the difference being not statistically significant, however, when the relative value of the S wave was analyzed as a function of the R wave, we observed that the difference becomes statistically significant, the S wave in chagasic mice represented a 48.78 ± 4.52 % and the healthy mice a 16.92 ± 2.20 % of R wave, being that the relative S amplitude was 2.87 times greater in chagasic mice (Table I).

To analyze the repolarization in the electrocardiographic traces, we assume that this phenomenon begins with the rise of the S to the J point, then traces the J wave and continue with a descent until a T wave with inferior convexity is traced, which can be continued or not with a superior convexity wave that has been called U wave (Figure 2). With this premise in mind we analyzed the morphological patterns of repolarization and have classified them into 5 types. Normal-like (Figure 2, Panel A), in this pattern all phenomena previously described except for the U wave were observed, T wave may be above or below the isoelectric line and tends to return to the isoelectric line; in leads II and AVF, T wave remains above the isoelectric line, whereas in lead I in 41.09% of healthy mice and in 4.46% of chagasic mice it remained below the isoelectric line delineating a negative T wave; normal like pattern was significantly associated with healthy mice (Table III). In the U wave pattern all phenomena described including a well-defined U wave was observed (Figure 2, Panel B), this pattern was significantly associated with chagasic mice (Table III). In the J wave absent pattern, ascent of S does not exceed the isoelectric line, observing the following variants: first, the isoelectric line is not reached and an ST depression is traced (Figure 2, Panels D and E), second the isoelectric line is reached and maintained flat (Figure 2, Panel F) and third, once the isoelectric line is reached a negative deflection (JT negative) was observed (Figure 2, Panel C); this pattern was significantly associated with the chagasic mice (Table III). In the delayed J wave ascent or plateau pattern, J wave peak was delayed because SJ slope decrease, also a presence of a plateau (ST elevation) was observed (Figure 2, Panels H and G, respectively); these were significantly associated with chagasic mice (Table III). In the TP segment elevation pattern the T wave is maintained above the isoelectric line until the P wave start, delineating a TP segment elevation (Figure 2, Panel E); this pattern was not present in healthy mice and it was significantly associated with chagasic ones (Table 3).

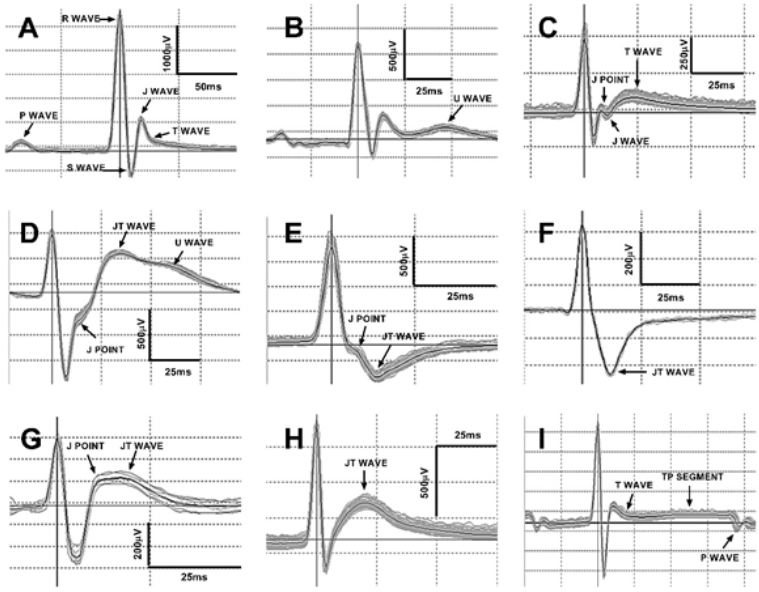

Figure 2: *Repolarization disturbances. Panel A shows a normal trace; panel B show a U wave and a decreased QRS complex; panel C shows a negative J wave follow by positive sustained T wave; panel D shows a negative J point follow by positive flattened JT wave and U wave; panel E shows a negative J point follow by negative JT wave; panel F shows negative JT wave; panel G shows a JT wave that lines a delayed plateau; panel H shows a delayed prolonged JT wave; and I wave shows a TP segment above isoelectric lines. All mice chagasic traces (B to G) show decreased QRS complex amplitude. Referential values of vertical and horizontal bars are indicated for each trace.*

Morphology	Healthy						Chagasic					
	DII		DIII		AVF		DII		DIII		AVF	
	n	%	n	%	n	%	n	%	n	%	n	%
Normal Like	59	80.8*	53	72.6*	60	81*	18	14.1	31	17.8	14	12.2
U wave	12	16.4	14	19.2	11	14.9	55	43.0*	56	32.2*	48	41.7*
J wave Absent	0	0	0	0	2	2.7	15	11.7*	41	23.6*	14	12.2*
Delayed J wave	0	0	6	8.2	1	1.4	33	25.8*	37	21.3*	31	27*
TP segment elevation	0	0	0	0	0	0	7	5.5*	9	5.2	8	6.9*
Total	73	100	73	100	74	100	128	100	174	100	115	100

Table III: *Ecg Repolarizarion Morphologies on Healthy and Chagsic Mice.*

The values of the amplitudes of J and T were significantly decreased in chagasic mice (J: 211.3 ± 13.50 and T: 53.55 ± 5.15 µV, respectively), in contrast to healthy mice (J: 438.5 ± 10.35 and T: 118.8 ± 6.06 µV, respectively) (Table I).

The decay of the J wave (T wave) could be mathematically adjusted to two components: a fast one (τ_1) and a slow one (τ_2); the fast component was significantly slower in chagasic mice (7.65 ± 0.36 ms) compared to healthy ones (4.09 ± 0.11 ms), while the slow component presented similar values in both groups. The contribution of both components to J wave amplitude descent was different in both groups of mice, the fast component was the predominant in healthy mice (90.51 ± 1.13%), and while the slow component traced the 48.05 ± 2.76% of the J wave descent in the chagasic mice. To corroborate these data, we measured normalized amplitudes of the J wave descent at 5, 10, 20, 40 and 60 ms; at all these times J wave amplitudes were higher in the chagasic mice (Table I).

Likewise, conduction and rhythm disorders were observed. In healthy mice, only tachycardia (13.5%), bradycardia (8.11%), atrial extrasystoles (6.8%), prolonged QRS (6.8%) and notched QRS (14.9%) were observed. In the chagasic mice, non-sinus rhythm (20.81%), nodal rhythm (15.6%), ventricular extrasystoles (15.6%) and ventricular first-degree atrioventricular block (52.61%) were only present and statistically associated with Chagasic mice. The atrial extrasystoles and the prolonged QRS interval were present in both groups but statistically associated to chagasic mice; while atrial supraventricular or ventricular tachycardia, atrial fibrillation, atrio-ventricular dissociation, atrial flutter, atrial ectopic, nodal extrasystoles, and ventricular atrial II and III degrees were present only in chagasic mice, but no statistical association was obtained because of the low frequency (between 0.6 and 4.6%) (see Table IV and Figure 3).

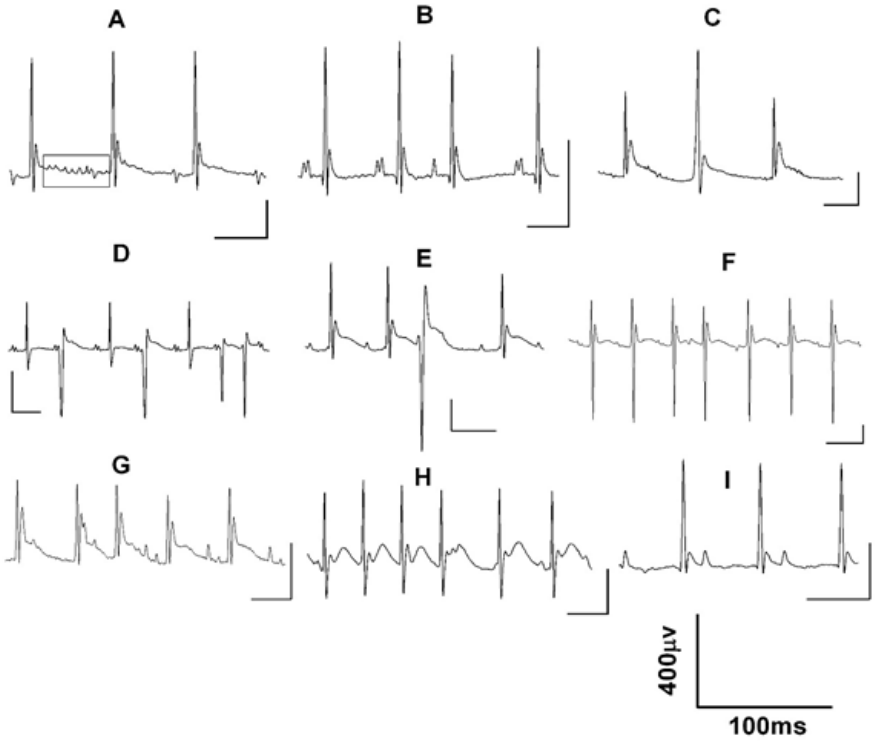

Figure 3: *Qualitative disorders. Panel A show an atrial flutter in the insert, biphasic p wave and prolonged T wave decay; panel B show an atrial extrasystole and bimodal p wave; panel C show a nodal extrasystole in the middle and prolonged T wave decay; panel D show multiple ventricular extrasistols, bimodal p wave and flattened T wave; panel E shows a ventricular extrasystole raising on U wave; panel F show a migratory pacemaker, U waves and increased S wave amplitude; panel G shows a typical atrial fibrillation with dominant p wave; panel H show atrial fibrillation and U waves; and panel I shows prolonged PR segment.*

Disorders	Healthy mice (n = 74)		Chagasic mice (n = 173)	
	Fr	%	Fr	%
Non sinusal rhythm	0	0	36	20.81*
Nodal rhythm	0	0	27	15.6*
Tachycardia	10	13.5	23	13.3
Supraventricular tachycardia	0	0	3	1.7
Ventricular tachycardia	0	0	1	0.6
Atrioventricular dissociation	0	0	8	4.6

Atrial Fibrillation	0	0	6	3.5
Atrial Flutter	0	0	1	0.6
Bradycardia	6	8.11	24	13.87
Atrial ectopics	0	0	8	4.6
Atrial extrasystoles	5	6.8	31	17.9*
Nodal extrasystoles	0	0	1	0.6
Ventricular extrasystoles	0	0	27	15.6*
I degree AV block	4	5.4	91	52.61*
II degree AV block	0	0	3	1.7
III degree AV block	0	0	1	0.6
Prolonged QRS segment	5	6.8	64	37,01*
Notched QRS	11	14.9	11	6.4

Table IV: *Rhytm and Conduction Disorders in Healthy and Chagasic Mice.*

Isoproterenol stress test was designed with the goal of inducing cardiac ischemia, by increasing myocardial work above energetic availability to suffice demand. In healthy mice isoproterenol initially increased the amplitude of the J wave with a delayed peak and decreased the descent, later progressively the J wave decreased in amplitude until becoming negative, to finally partially recovering its original morphology. These changes in the J wave were accompanied by an increase in the S wave amplitude. In the chagasic mice, the same alterations were observed, however, the S wave became progressively wider and the J point lost its definition (Figure 4).

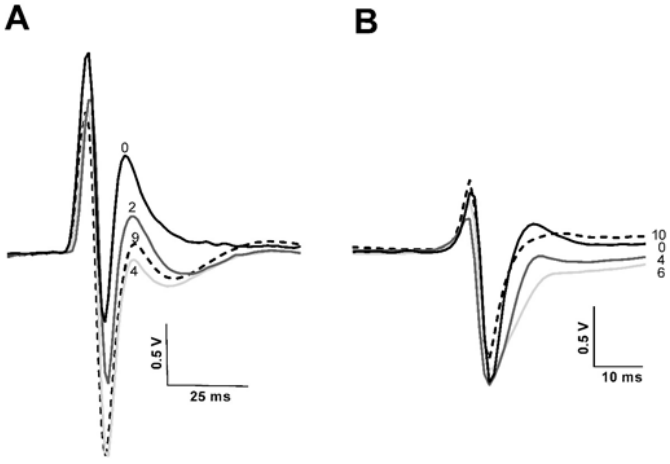

Figure 4: *Isoproterenol effect on ECG traces of healthy and chagasic mice. Isoproterenol 1 mg/Kg administered to healthy mice induced a decrease in the J wave amplitude between 2 and 4 min after administration; recovery was initiated after 9 min; also S wave amplitude increased (panel A). Chagasic mice show similar pattern, but S wave rise was amply delayed and amplitude was decreased.*

Histopathological features are displayed in Figure 5. Severe myocarditis, with lymphomononuclear infiltration, miocytolisis and fiber disorganization was observed during acute *Trypanosoma cruzi* acute infection. A more detailed analysis revealed a preferment inflammatory pattern location in heart base (upper panel, left) and right ventricle (upper panel, right), with lesser compromising in left ventricle (lower panel, left). Inflammatory infiltrate associated to cardiac microvasculature (arrows) and fibrine patches within intramural coronary vessels were observed, suggesting myocardial ischemic disorders.

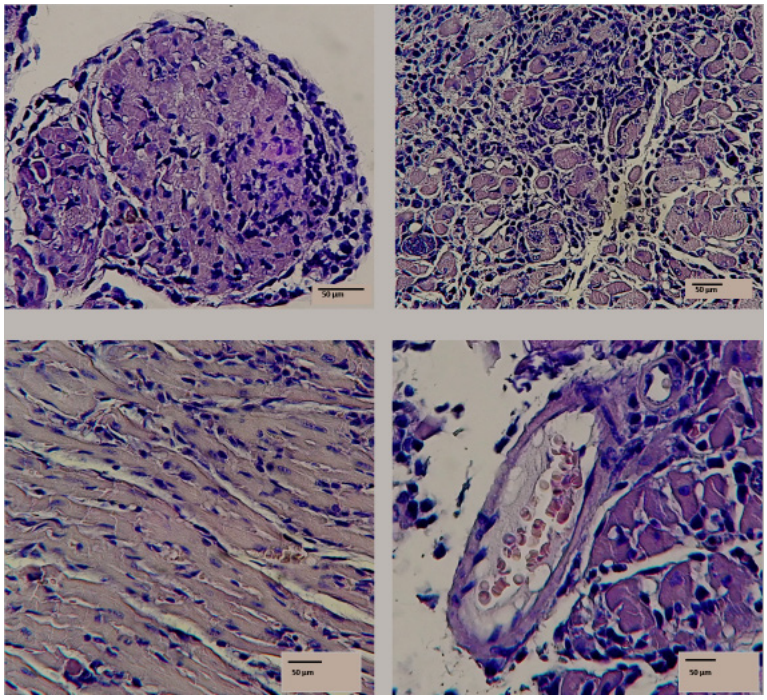

Figure 5: *Histopathological features. Different details of cardiac inflammation are shown in H&E stained tissue slides. Atrial and right ventricular samples are shown at upper left and right panels, respectively; while left ventricle and vascular vessels are shown at bottom left and right pannels, respectively. In this figure we illustrate that inflammation is more severe in atria and left ventricle, as compared with right ventricle. Also we illustrate the presence of thrombi "(***)" into the vascular vessels that explain ischemia phenomenon. Arrows details pericitary inflammation. Horizontal bars are equivalent to 50 µm for atria and vascular vessels panels and 100 µm for right and left ventricles panels.*

Discussion

In the present work we analyzed the electrocardiographic data of NMRI mice with acute chagasic myocarditis and compared them with the data of healthy mice, in order to contrast and validate the normal and abnormal electrocardiogram parameters in this mice strain. We found that the typical electrocardiographic morphology statistically associated with healthy mice was a positive monophasic or biphasic (positive deflection followed by a negative inflection) P wave, a QRS complex with a practically imperceptible Q wave, a positive R wave, and a S wave absent or negative; the QRS complex ends at J point, then a J wave is traced and continued with a descent until a concave or convex T wave could be defined, T wave may be above or below the isoelectric line and had a tendency to return to the isoelectric line. Abnormal electrocardiographic morphology statistically associated with the chagasic mice were negative, bimodal (humped) positive or double synchronous non identical P waves; the QRS complex was followed by a J wave with a delayed rise that had a tendency to outline a plateau that can be considered an ST elevation or by defect the J wave did not exist but instead formed an ST depression; the presence of a U wave and a TP segment above the isoelectric line are pathological and associated to the chagasic mice.

The P wave in the ECG represents atrial depolarization, normally the right atrium depolarizes slightly earlier than left atrium since the depolarization wave originates in the sinoatrial node. The depolarization front is carried through the atria along semi-specialized conduction pathways including Bachmann's bundle resulting in uniform shaped positive waves in bipolar leads and AVF. In the present study, the ECG of healthy mice were significantly associated with positive P waves predominant in lead II, however, in the AVF lead the positive P-wave had a frequency similar to positive-negative biphasic P waves; whereas in lead III a negative P wave was predominant.

These results can be explained by the axis of the P wave, which would have an axis between 0 and + 30°, which would indicate a greater horizontality of the axis with respect to the values reported in humans, between +45 and +75° [20].

The positive bimodal P wave and the double P wave were statistically associated with the chagasic mice and would therefore represent pathological traces. In humans the bimodal P wave is also referred to as a double peak (bifid) P wave similar to a camel hump, it is generally observed in lead II, being a sign of left atrial enlargement [20]. The constant double P wave has not been described as a particular entity in the scientific literature, and it would be difficult to interpret it sustained in the bibliographical reports, however, it could mean a dissociation in the depolarization between the right and left atria by atrial conduction disorders in the Bachmann's bundle, an atypical atrial flutter, or an ectopic pacemaker synchronized with the sinus node. Atrial ectopy is supported in the present study by observing multiple atrial ectopic, atrial flutter and atrial fibrillation in the chagasic animals. Intra- and interatrial conduction abnormalities delays disrupt (spatial and temporal dispersion) electrical activation, thus promoting the initiation and perpetuation of reentrant circuits associated with atrial fibrillation, typical or atypical flutter [21].

The Q wave is difficult to assess because it manifests inconstantly in lead I and is not observed in the other leads tested in the present work. In humans the Q wave can be observed in leads I, II, aVL, V5 and V6 and represents an initial ventricular activation on the left side of the ventricular septum and the vector goes to the right. Unlike in humans, in mice the myocardium at the top of the septum is in contact with the His bundle, in consequence the onset of ventricular activation occurs at the top of the septum and the electrical vector goes to the apical region of the heart and no Q wave should be traced [4]. However, in murine models, as well in humans the Q wave becomes visible when a transmural infarction is stablished [4,5,22].

The main finding in this work regarding the R wave was that its amplitude was significantly decreased in the mice with chagasic cardiomyopathy. This may be a reflection of a loss of the heart's ability to generate voltage, due to the loss of myocardial function due to inflammation, parasitic invasion, hypoxia and myocytolysis, all of which encompass myocarditis. Similar findings have been reported in mice with myocarditis induced by encephalomyocarditis virus, where serial electrocardiograms revealed that the sum of the QRS voltages increased on day 3 and then decreased on days 7 to 9 [23].

S waves have been related to a depolarization of the Purkinje fibers in posterodiaphragmatic wall [24], its amplitude in humans is increased in left ventricular hypertrophy in leads III, AVF and precordial V1, V2 and V3. In the present paper we did not observe statistically significant differences in the absolute amplitude of S wave when comparing the healthy mice with the chagasic ones; however, when normalizing the value of the absolute amplitude in comparison to the R amplitude, we observed that the normalized amplitude of the chagasic mice becomes significantly greater. This can be explained by a diffuse myocarditis with deviation of the axis to the left by compensatory hypertrophy of the left ventricular posterodiaphragmatic wall in the chagasic mice. A diffuse myocarditis, plus intense myocardial hypertrophy, damage and fibrosis, in the presence of very few *Trypanosoma cruzi forms*, are the histopathological hallmarks of chronic chagasic cardiomyopathy [25].

In the present work we observed that the absent J wave pattern was associated with chagasic mice, this electrocardiographic pattern with inversion or flattened T wave would indicate the existence of subepicardial ischemia and/or right ventricle hypertrophy [26]. This is sustained by the histopathological findings, where the inflammation process associated with coronary microvasculature was more intense in right ventricle and in subepicardial areas, in comparison to the septum, left ventricle, and endocardial areas, respectively; but the existence of severe disorders of the microvasculature in chagasic myocardiopathy would speak in favor of ischemia [17]; concurring with this, in the present work fibrine patches were detected in intramural coronary vessels.

We also observed positive waves (U waves) after T waves, U wave patterns was associated to chagasic mice and therefore must represent pathological phenomena. Different theories have been raised to explain the U wave, including delayed repolarization of the ventricular septum, papillary muscles and Purkinje system, negative afterpotentials, early or delayed after depolarizations and mechano electrical feedback [references in 27]. However, since the chagasic mice exhibited relatively larger S wave amplitude, which would imply

a relative hypertrophy of the posterolateral walls of the left ventricle, then the charge displaced by the Purkinje fibers of these areas would be larger and could trace a U wave. Another explanation could be made based on the work of Liu et al, 2003 [9], who demonstrated that the experimental manipulation of murine transmural gradients by shortening epicardial monophasic after potentials results in a positive murine late T wave, however, this explanation would imply that epicardial action potentials are shortened in chagasic myocarditis, a fact that is not physiopathologically plausible, since inflammation and ischemia observed in epicardial areas, must affect cardiac repolarization and QT intervals which on the contrary were significantly prolonged.

In the present work, isoproterenol initially induced an increase and widening of the JT wave, reflecting a subendocardial ischemia, and then a negative J wave, reflecting subepicardial ischemia. The flattening of the JT wave or its inversion were preferentially observed in chagasic mice, confirming that in chagasic cardiomyopathy there are ischemic disorders, which are physiopathologically related to disorders in the coronary microvasculature associated with elevated levels of catecholamines. In these mice, the application of isoproterenol aggravated repolarization disorders, with a widest S wave due to a decrease in the ascent slope, disappearance of the J point and a more negative JT wave. The decrease in the slope of the S wave rise would indicate that this portion of the wave forms part of the rapid repolarization of the left ventricle and together with the disappearance of the J point and a more negative JT wave would represent electrocardiographic signs indicative of ischemic dysfunction. Isoproterenol induces repolarization disorders have been electrocardiographically visualized as an inversion of the J wave [28].

Finally we must make clear that the electrocardiographic records were performed under anesthesia with pentobarbital and ketamine. The main effects of this anesthesia were to reduce chronotropism and dromotropism, in fact the deeper the anesthesia, these effects were more visible. Healthy mice required more doses to achieve full anesthesia (between 30 and 40 mg/kg of each drug) compared to chagasic mice requiring 25 mg/kg of each drug, however, in healthy mice we did not observe rhythm or conduction disturbances. Nevertheless, there is a point that was not addressed in this work, which is the sensitivity of chagasic mice to anesthesia on the cardiac domain.

Conclusion

The relevance of the present paper in a translational context is to provide valuable quantitative and qualitative data that could allow researchers dedicated to the study of cardiac pathologies in mice to discern the differences in the electrocardiogram between pathological and normal records and thus to visualize electrocardiographically the pertinence of their mice model. On the other hand, the data presented here will enable cardiologists to visualize the correspondence between the murine and human electrocardiographic records, both in normal and physiological conditions, creating a basis for translating the data obtained in mice for their application in humans.

Here, we clearly illustrate by contrast analyses between electrocardiographic data from healthy mice against NMRI mice with chagasic myocardiopathy, the profile of electrocardiographic traces that should be considered as a pathological at least in this species, which can be systemized as follows:

1. Atrial disturbances: negative, bimodal or double dissimilar P wave;
2. Myocarditis reflected as decrease of the amplitude of QRS complex (mainly R wave) and lengthening of PR, QRS and QT intervals.
3. Ventricular repolarización disorders visualized as a decrease in the S wave rise, a plateau or otherwise flattening on J wave, TP segment above the isoelectric line and inversion of the J wave;
4. U waves could represent early after depolarization.

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Conflict of interest

We do not have any financial interest or any conflict of interest to declare.

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