Left Atrial and Left Ventricular Diastolic Function in Chronic Chagas Disease

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Background: Left atrial (LA) and left ventricular (LV) diastolic function analysis can yield new strategies to recognize early cardiac involvement and prognostic indicators in Chagas disease.

Methods: Patients with Chagas disease with the indeterminate (n = 69) or with the cardiac form (32 with changes limited to electrocardiography [stage A], 25 with changes in LV systolic function but no heart failure [HF; stage B], and 26 with HF) underwent evaluation of LV diastolic function (mitral inflow, pulmonary vein flow, color M-mode echocardiography, and tissue Doppler analysis), and LA function by three-dimensional echocardiography and strain analysis and were prospectively followed for the occurrence of clinical events. Echocardiograms were also obtained from 32 controls.

Results: LV diastolic dysfunction was gradually more prevalent and severe across groups from patients with the indeterminate form of Chagas disease to patients with HF. Tissue Doppler was the best tool to demonstrate the worsening of LV diastolic function across the groups (E′ velocity: controls, 12.6 ± 2.3 cm/sec; patients with the indeterminate form, 12.1 ± 3.1 cm/sec; stage A, 10.3 ± 2.9 cm/sec; stage B, 8.3 ± 2.8 cm/sec; patients with HF, 5.6 ± 1.9; $P < .0001$). Although maximum LA volume was increased only in patients with HF, minimum LA volume (controls, 8 ± 2 mL/m^2; patients with the indeterminate form, 8 ± 2 mL/m^2; stage A, 9 ± 3 mL/m^2; stage B, 11 ± 4 mL/m^2; patients with HF, 27 ± 17 mL/m^2; $P < .0001$) and precontraction LA volume (controls, 11 ± 3 mL/m^2; patients with the indeterminate form, 12 ± 3 mL/m^2; stage A, 13 ± 4 mL/m^2; stage B, 16 ± 5 mL/m^2; patients with HF, 32 ± 19 mL/m^2; $P < .0001$) were increased in all cardiac form groups. LA conductive function was depressed in all cardiac form groups, while LA contractile function was depressed only in patients with HF. Cox proportional-hazards regression analysis revealed that end-systolic LV diameter (hazard ratio, 1.6; 95% confidence interval, 0.9–2.8; $P = .09$), E′ velocity (hazard ratio, 0.5; 95% confidence interval, 0.3–0.8; $P = .001$), and peak negative global LA strain (hazard ratio, 1.21; 95% confidence interval, 1.02–1.4; $P = .03$), were independent predictors of clinical events.

Conclusions: LV diastolic dysfunction was found in all forms of chronic Chagas disease, including those without LV systolic dysfunction. LV diastolic dysfunction may contribute to changes in LA volume and conductive function found in early stages of the cardiac form. Both LV diastolic function and LA contractile function were independent predictors of clinical events. (J Am Soc Echocardiogr 2013;26:1424-33.)

Keywords: Two-dimensional strain, Left atrial function, Left ventricular diastolic function, Real-time three-dimensional echocardiography, Chagas disease

About 10 million people worldwide are chronically infected by *Trypanosoma cruzi,* mostly in Latin America. However, Chagas disease has been increasingly detected in other countries in the Americas, the western Pacific region, and Europe[1] because of population migration.[2]

The consequences of Chagas disease on left ventricular (LV) systolic function are well described, but studies regarding LV diastolic function and left atrial (LA) volume and function in Chagas disease are still limited. LA index is a recognized prognostic marker in many conditions, such as heart failure (HF),[3] atrial fibrillation,[4] and Chagas

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Abbreviations

CI = Confidence interval

ELISA = Enzyme-linked immunosorbent assay

ε = Strain

HF = Heart failure

LA = Left atrial

LV = Left ventricular

RT3DE = Real-time three-dimensional echocardiography

RV = Right ventricular

STE = Speckle-tracking echocardiography

2D = Two-dimensional

VTI = Velocity-time integral

disease.[5] Moreover, LA function was described as a prognostic indicator for atrial arrhythmias[6] and for in-hospital mortality after myocardial infarction.[7] LA function may emerge as an important component in the evaluation of Chagas disease because atrial arrhythmias and HF are common complications of Chagas heart disease.[8] New technologies allow noninvasive measurement of the components of LA function (contractile, conduit, and reservoir). Those techno-logies include real-time three-dimensional echocardiography (RT3DE) and two-dimensional (2D) strain (ε) analysis by speckle-tracking echocardiography (STE). STE may allow a more direct assessment of LA myocardial contractility and passive deformation, and reference values have been already published.[9-12]

There are few reports addressing LV diastolic function in Chagas disease.[13-18] A large retrospective study evaluated only LV mitral inflow but identified LV diastolic dysfunction in the chronic indeterminate form and in all stages of Chagas heart disease.[13] In another study, the severity of the LV diastolic dysfunction in Chagas disease was strongly correlated with LA dimension and LV dimensions and ejection fraction.[14] Tissue Doppler–derived parameters were also described as survival predictors in Chagas disease.[19] Moreover, LA function is closely related to LV diastolic function,[12] and a thorough evaluation of LV diastolic function and LA function including patients with different forms of Chagas disease is still missing. Therefore, our aim was to evaluate LV diastolic function and LA function in patients with Chagas disease in different forms and at different stages to identify early changes in these parameters and their prognostic value.

METHODS

Patients

Patients with chronic Chagas disease, diagnosed by two different serologic tests (enzyme-linked immunosorbent assay [ELISA] and immunofluorescence), between 18 and 60 years of age were prospectively and consecutively invited to participate in this study. The cutoffs used for ELISA were previously published.[20] Results of immunofluorescence were considered positive whenever fluorescence was observed at dilutions > 1:40. The study was approved by the local ethics committees (no. 0059.0.009.000-09) and conformed to standards currently applied by the Brazilian National Committee for Research Ethics. All subjects gave written informed consent before their participation.

Control subjects were recruited among those referred to our institution for Chagas disease diagnosis who tested negative for Chagas disease on the two serologic tests; had no known diseases; had normal results on physical examination, electrocardiography, and echocardiography; had normal LV systolic function; and had no significant valvar disease.

The age limit of 60 years was arbitrarily chosen because of the association between age and diastolic dysfunction.[21] Patients were classified at the time of their enrollment in the study according to the current Brazilian Chagas disease consensus[22] as indeterminate (no evidence of cardiac involvement), stage A (no HF symptoms with isolated electrocardiographic changes), stage B (no HF symptoms with segmental or global LV systolic dysfunction), stage C (symptomatic HF), or stage D (end-stage HF). For study analysis, stage C and D patients were grouped together.

Echocardiography

Studies were performed using a phased-array ultrasound system (Vivid 7; GE Medical Systems, Milwaukee, WI) equipped with an M4S phased-array and a 2- to 4-MHz 4 matrix-array transducers. Echocardiograms were reviewed offline using EchoPAC PC version 108.1.12 (GE Medical Systems).

Cardiac dimensions and Doppler measurements were obtained in accordance with American Society of Echocardiography recommendations.[23] M-mode echocardiography was used to measure LA diameter and LV end-diastolic and end-systolic diameters. Two-dimensional LV and LA volumes were determined using the modified Simpson's rule, with images obtained from apical four-chamber and two-chamber views. Pulsed-wave Doppler was performed in the apical four-chamber view. From transmitral recordings, the peak early (E) and late (A) diastolic filling velocities, E/A ratio, E-wave deceleration time, velocity-time integral (VTI) of the E wave (VTI_E), A-wave VTI (VTI_A), and LA filling fraction [$VTI_A/(VTI_E + VTI_A)$] were obtained. From pulmonary vein velocities obtained at the right upper pulmonary vein, the following measurements were taken: peak S-wave inflow velocity during ventricular systole, peak D-wave inflow velocity during the early phase of ventricular diastole and the corresponding S/D ratio, and peak reversed atrial wave (Ar) velocity during LA contraction. Isovolumic relaxation time was measured from continuous-wave Doppler obtained in the apical long-axis view. Propagation velocity (Vp) of early LV inflow was measured using color M-mode echocardiography from the apical four-chamber view. Right ventricular (RV) systolic pressure was derived from continuous-wave Doppler interrogation of tricuspid regurgitation, in accordance with American Society of Echocardiography recommendations.[24] RV systolic function was evaluated by measuring the peak systolic myocardial velocity (RV S′) of the lateral tricuspid annulus and the tricuspid annular plane systolic excursion, as recommended.[24]

Tissue Doppler of the mitral annulus was obtained at the septal and lateral positions. Values shown for peak systolic myocardial velocity (S′) and peak early (E′) and late (A′) diastolic myocardial velocities are averages of the values obtained at the septal and lateral positions.

Two-Dimensional ε Analysis

Two-dimensional speckle-tracking software (EchoPAC PC) was used to calculate LV longitudinal, circumferential, and radial ε; LV torsion and twist, RV longitudinal ε; and LA ε. All 2D clips analyzed were acquired at high frame rates (>60 frames/sec).

LA ε Analysis. LA ε was determined as previously described[12] using images obtained in the apical four-chamber, two-chamber, and three-chamber views. Regarding the three-chamber view, we included only the inferoposterior wall, because the opposing wall includes the ascending aorta.[10] The onset of the P wave was used as the reference

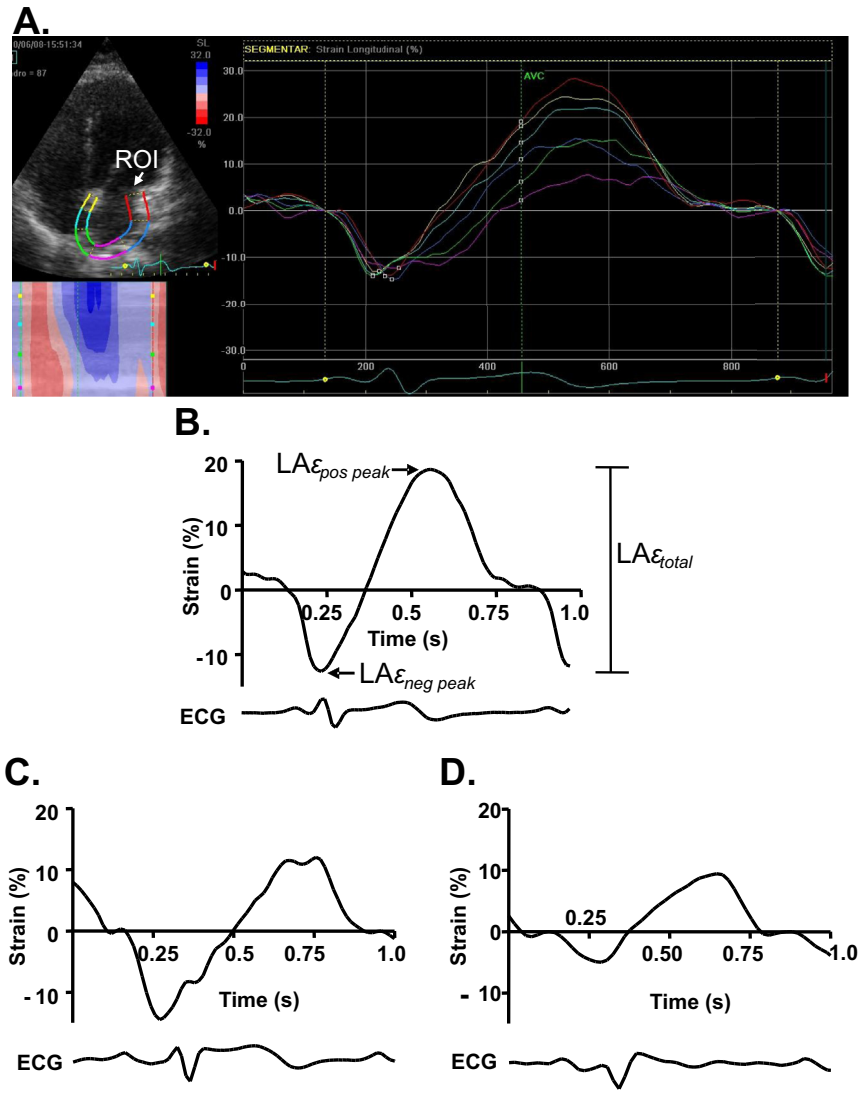

Figure 1 Two-dimensional LA ε in Chagas disease. **(A)** EchoPAC PC software was used to analyze the apical four-chamber view obtained from a control individual. The onset of the P wave was used as the reference point for the calculation of LA ε. The LA endocardial surface was manually traced using a point-and-click approach. An epicardial surface tracing was automatically generated by the system, creating a region of interest (ROI), which was manually adjusted to cover the full thickness of the myocardium. The LA view was divided into six segments, and curves corresponding to LA ε for each of these segments are displayed on the right side of the panel. **(B)** Average LA ε curve obtained after averaging six curves on the panel shown in **(A)**. Note the LA $\varepsilon_{pos\ peak}$, which corresponds to LA conduit function; LA $\varepsilon_{neg\ peak}$, which corresponds to LA contractile function; and LA ε_{tot}, which corresponds to LA reservoir function. **(C)** Average LA ε curve of a patient at stage A depicting reduced LA $\varepsilon_{pos\ peak}$ and similar LA$\varepsilon_{neg\ peak}$, which demonstrated depressed LA conductive function and maintenance of LA contractile function. **(D)** Average LA ε curve of a patient at stage C depicting reduction in both LA $\varepsilon_{pos\ peak}$ and LA$\varepsilon_{neg\ peak}$, which demonstrated depressed LA conductive and contractile function. *ECG*, Electrocardiogram.

point for LA ε calculation, which enabled the recognition of peak positive global LA ε ($\varepsilon_{pos\ peak}$), which corresponded to LA conduit function; peak negative global LA ε ($\varepsilon_{neg\ peak}$), which corresponded to LA contractile function; and the sum of those previous values (total global LA ε [ε_{tot}]), which corresponded to LA reservoir function. The final LA ε values were the averages of the values obtained for each apical view (Figure 1).

Two-Dimensional LV and RV ε Analysis. LV longitudinal, circumferential, and radial ε were calculated as previously described.[25] Electrocardiographic R-wave onset was used as the reference point. LV circumferential and radial ε was analyzed in short-axis views at the basal level, defined by visualization of the tips of the mitral valve; at the midlevel, defined by visualization of the papillary muscles; and at the apical level, defined as the LV cavity with no visible papillary muscles and a minimally visible right ventricle. LV longitudinal ε was analyzed in the four-chamber, two-chamber and three-chamber views. Global ε in each view was obtained by averaging the six regional ε curves obtained for each LV view. Peak global LV circumferential and radial ε values were the averages of the peak averages for global LV circumferential and radial ε obtained in each short-axis views. Peak global LV longitudinal ε was calculated

similarly using long-axis views. In case tracking quality was not good in two segments of the same acoustic window, that view was excluded from global LV ε calculation. RV longitudinal ε was calculated similarly to LV longitudinal ε using four-chamber apical views.

LV Torsion Calculation. LV torsion and twist were calculated as previously described.[26] LV twist was defined as the net difference of LV rotation between the apical and basal short-axis planes obtained from speckle-tracking echocardiographic analysis and LV torsion as LV twist divided by end-diastolic LV longitudinal length. LV rotation was defined as angular displacement of the LV about its central axis in the short-axis image. These values were expressed as degrees. Counterclockwise LV rotation as viewed from apex was expressed as a positive value. The same short-axis and apical views used for LV ε analysis were used to calculate LV rotation using the same approach.

LA and LV Volume and Function Analysis by RT3DE

RT3DE was performed in apical views. Three-dimensional LA images were taken using the full-volume method during end-expiration. Offline software (EchoPAC PC) was used for analyzing LA three-dimensional images, as previously described.[27] Time-volume curves were obtained and used to determine maximum LA volume, minimum LA volume, and LA volume before LA contraction (precontraction LA volume). The following indexes of LA function were calculated, according to previous studies.[27] Total LA emptying fraction was calculated as [(maximum LA volume − minimum LA volume)/maximum LA volume] × 100. Active LA emptying fraction was calculated as [(precontraction LA volume − minimum LA volume)/precontraction LA volume] × 100. Passive LA emptying fraction was calculated as [(maximum LA volume − precontraction LA volume)/maximum LA volume] × 100. LV volume was measured using a similar approach, as previously described.[28] Time-volume curves were obtained and used to determine LV end-diastolic and end-systolic volumes and three-dimensional LV ejection fraction.

Survival Analysis

Patients were followed for the occurrence of a combined end point of all-cause mortality, stroke, heart transplantation, atrial fibrillation, or admission for worsening HF or cardiac arrhythmias. A multivariate Cox proportional-hazards regression analysis adjusted for age and sex was performed to identify independent predictors of the combined end point. Variables were entered in the model if their associated *P* values were <.05 and removed from the model if their associated *P* values were >.10.

Statistical Analysis

Calculations were done using MedCalc 12.5.0.0 (MedCalc Software, Mariakerke, Belgium). Continuous variables are expressed as mean ± SD and discrete variables as percentages. All echocardiographic variables passed standard tests of normality (Kolmogorov-Smirnov test) allowing the use of parametric tests. Data between groups were compared using one-way analysis of variance followed by Student-Newman-Keuls post hoc analysis. Correlation between LA function and LV systolic and diastolic function parameters was analyzed using stepwise multiple regression analysis. Values obtained using two different techniques were compared using intraclass correlation coefficients. Interobserver and intraobserver agreement for global LA ε and LA volumes was determined after offline reanalysis of recorded clips of 14 randomly selected subjects and assessed by Bland-Altman analysis.[29] *P* values ≤ .05 were considered significant.

RESULTS

Subjects Characteristics

A total of 251 patients with Chagas disease were enrolled in the study between March 2010 and June 2012. Of these, 99 were excluded from analysis because of hypertension ($n = 55$), diabetes ($n = 10$), permanent pacemakers ($n = 14$), coronary artery disease ($n = 6$), atrial fibrillation ($n = 3$), rheumatic heart disease ($n = 1$), congenital heart disease ($n = 1$), associated digestive form of Chagas disease ($n = 3$), pregnancy ($n = 1$), associated moderate to severe systemic disease ($n = 3$), or inadequate imaging quality ($n = 2$).

All patients tested positive on both ELISA and immunofluorescence. The reactive indexes obtained on ELISA ranged from 2.0 to 10.5, and immunofluorescence ranged from 1:80 to 1:2,560. All patient groups had similar ages. Body mass indexes were reduced in patients at stages C and D. There was a female predominance among stage A patients. Electrocardiographic changes were predominant among patients with the cardiac form, as expected (Table 1).

Chamber Diameters and Systolic Function

LV and LA diameters were increased in patients with more advanced stages of the cardiac form. LV systolic function was decreased in patients at stages B, C, and D, as assessed by LV ejection fraction and LV S′. RV systolic dysfunction and pulmonary hypertension were present only in stage C and D patients, as assessed by RV S′, RV ε, and tricuspid annular plane systolic excursion (Table 2). Mild functional mitral regurgitation was present in 25 patients (three indeterminate, five stage A, five stage B, 10 stage C, and two stage D), while functional mitral regurgitation was classified as moderate in four patients at stage C and two at stage D and as severe in two patients at stage D.

LV Diastolic Function

Mitral flow and tissue Doppler were obtained from all patients. Except for one indeterminate patient, two patients at stage A, one patient at stage C, and two patients at stage D, pulmonary vein flow was obtained from all patients. Except for three controls, one patient with the indeterminate form, and two patients at stages A, C, and D, Vp was obtained from all patients. LV diastolic dysfunction was present in all Chagas disease patient groups. However, it was gradually more prevalent and more advanced from patients with the indeterminate form to patients in the stages C and D of the cardiac form (Table 3).

Tissue Doppler was the best tool to demonstrate the gradual worsening in LV diastolic function across the groups. Although E/A ratio was significantly increased only in patients at stages C and D, E′ was progressively lower from patients at stage A to patients at stages C and D. E/E′ ratio was increased in indeterminate patients compared with controls and increased progressively across groups to patients at stages C and D (Figure 2). E′/A′ ratio was decreased in patients at stages A and B but improved toward normal in patients at stages C and D because of the decrease in A′ observed in those patients. Isovolumic relaxation time was increased in patients at stage B and was further increased in those at stages C and D. Except for S and

Table 1 Clinical characteristics of subjects

Variable	Controls (*n* = 32)	Indeterminate (*n* = 69)	Stage A (*n* = 32)	Stage B (*n* = 25)	Stages C and D (*n* = 26)
Age (y)	44 ± 7	45 ± 9	48 ± 8	48 ± 10	49 ± 8
Men	44%	48%	34%	60%	65%
BMI (kg/m^2)	26 ± 4	26 ± 4	25 ± 4	26 ± 4	23 ± 4*,†,‡,§
ECG					
RBBB	0%	0%	78.1%*,†	68%*,†	34.6%*,†,‡,§
LBBB	0%	0%	3.1%	4%	7.7%
LAHB	0%	4.3%	50%*,†	52%*,†	65.4%*,†
Primary repolarization changes	0%	0%	37.5%*,†	52%*,†	42.3%*,†
Medications					
Carvedilol	0%	0%	0%	24%	88%
ACE inhibitor	0%	0%	0%	32%	81%
ARB	0%	0%	0%	0%	15%
Digoxin	0%	0%	0%	0%	31%
Spironolactone	0%	0%	0%	4%	92%
Furosemide	0%	0%	0%	0%	88%
Amiodarone	0%	0%	3%	0%	15%

Data are expressed as mean ± SD or as percentages.
ACE, Angiotensin-converting enzyme; *ARB*, angiotensin receptor blocker; *BMI*, body mass index; *ECG*, electrocardiography; *LAHB*, left anterior hemiblock; *LBBB*, left bundle branch block; *RBBB*, right bundle branch block.
*P < .05 versus controls.
†P < .05 versus indeterminate.
‡P < .05 versus stage A.
§P < .05 versus stage B.

Table 2 Chamber diameters and systolic function

Variable	Controls (*n* = 32)	Indeterminate (*n* = 69)	Stage A (*n* = 32)	Stage B (*n* = 25)	Stages C and D (*n* = 26)
Left atrium (cm)	3.4 ± 0.5	3.5 ± 0.4	3.6 ± 0.4	3.8 ± 0.4*,†,‡	4.4 ± 0.8*,†,‡,§
LVd (cm)	5.0 ± 0.4	5.0 ± 0.4	5.2 ± 0.5	5.7 ± 0.6*,†,‡	6.7 ± 0.7*,†,‡,§
LVs (cm)	3.1 ± 0.4	3.0 ± 0.4	3.2 ± 0.5	4.1 ± 0.8*,†,‡	5.7 ± 0.9*,†,‡,§
LV ejection fraction (%)	68 ± 6	68 ± 6	67 ± 7	55 ± 9*,†,‡	34 ± 12*,†,‡,§
LV mass (g/m^2)	59 ± 11	64 ± 17	67 ± 19	78 ± 19*,†,‡	107 ± 29*,†,‡,§
LV S′ (cm/sec)	9.2 ± 1.6	9.1 ± 1.6	9.0 ± 2.1	6.7 ± 1.2*,†,‡	5.0 ± 1.3*,†,‡,§
RV S′ (cm/sec)	14.3 ± 2.2	14.2 ± 2.1	13.7 ± 2.3	12.7 ± 2.1*,†	10.7 ± 3.2*,†,‡,§
RV ε (%)	−22 ± 2	−22 ± 4	−22 ± 3	−21 ± 3	−15 ± 6*,†,‡,§
TAPSE (mm)	24 ± 4	24 ± 3	25 ± 5	24 ± 5	19 ± 7*,†,‡,§
RVSP (mm Hg)	28 ± 3	28 ± 4	28 ± 4	32 ± 7	44 ± 19*,†,‡,§

Data are expressed as mean ± SD.
LVd, LV end-diastolic diameter; *LVs*, LV end-systolic diameter; *RVSP*, RV systolic pressure; *TAPSE*, tricuspid annular plane systolic excursion.
*P < .05 versus controls.
†P < .05 versus indeterminate.
‡P < .05 versus stage A.
§P < .05 versus stage B.

Table 3 Frequency of LV diastolic patterns in the studied groups

Pattern	Controls (*n* = 32)	Indeterminate (*n* = 69)	Stage A (*n* = 32)	Stage B (*n* = 25)	Stages C and D (*n* = 26)
Normal	31 (97%)	62 (89.8%)	16 (50%)	9 (36%)	2 (7.7%)
Delayed relaxation	1 (3%)	6 (8.7%)	11 (34.4%)	9 (36%)	5 (19.2%)
Pseudonormal	0	1 (1.5%)	5 (15.6%)	5 (20%)	12 (46.2%)
Restrictive	0	0	0	2 (8%)	7 (26.9%)

Ar velocities in stage A patients, average values for pulmonary vein parameters did not present significant differences across the groups. Although Vp was significantly depressed in patients at stages A, C, and D, E/Vp was increased in all Chagas disease groups and further increased in stage C and D patients. Untwist was decreased in stage B, C, and D patients compared with all other groups (Table 4).

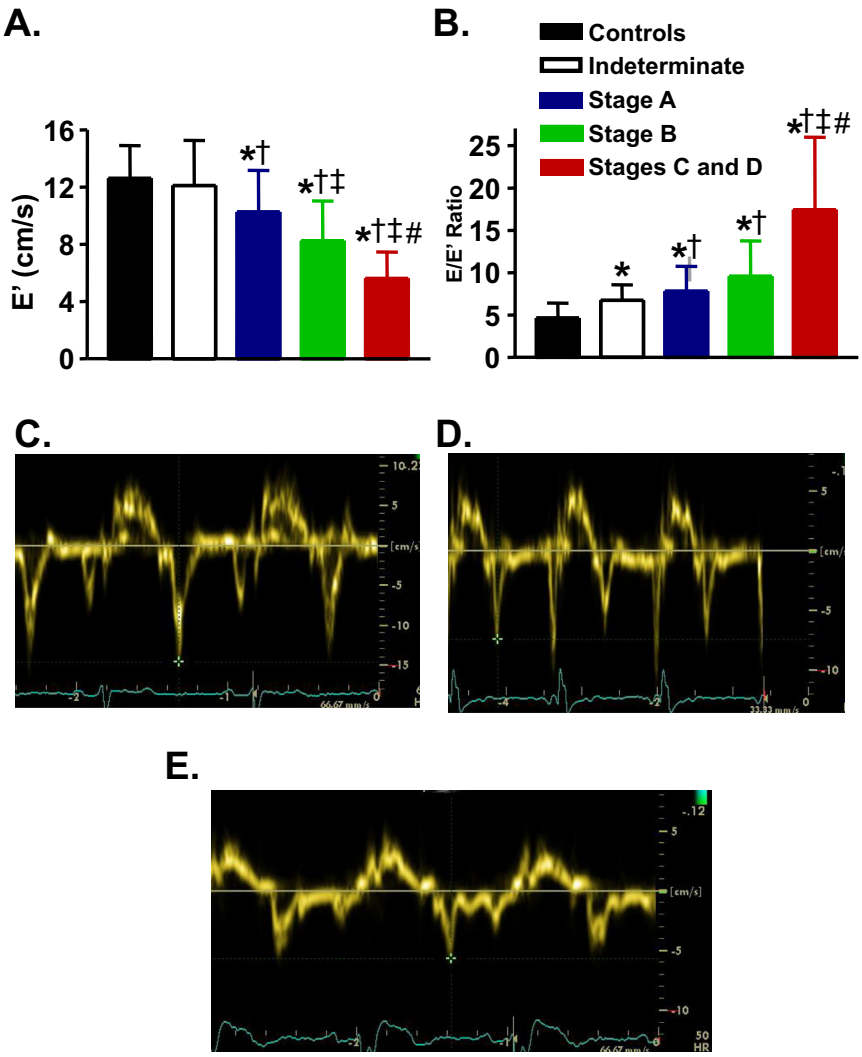

Figure 2 LV diastolic dysfunction in Chagas disease. **(A)** Bar graph depicting the gradual decline in E′ velocity across stages of the cardiac form. **(B)** Bar graph depicting increased E/E′ ratio in all Chagas disease groups compared with controls. E/E′ ratio is also higher in all stages of the cardiac form compared with the indeterminate group and presents a further increase in patients with HF (stages C and D) compared with all other groups. Examples of tissue Doppler tracings from control subject **(C)** and patients in stage A **(D)** and stage C **(E)** of the cardiac form. Note the gradual decline in E′ velocity across the examples, while A′ velocity is reduced only in the patient at stage C **(E)**.

LA Volume and Function by RT3DE and ε Analysis

Except for one control and one patient at stage A, real-time three-dimensional echocardiographic images of sufficient quality to determine LA volumes were obtained from all other patients. Although maximum LA volume was increased compared with other groups only in stage C and D patients, minimum and precontraction LA volumes were already increased compared with controls in stage A and B patients. Minimum and precontraction LA volumes were also progressively larger in stage B, C, and D patients compared with other groups of patients. Total LA emptying fraction was decreased in stage A and B patients compared with patients with the indeterminate form and in stage B patients compared with controls. Total LA emptying fractions were also decreased in the group of patients with HF compared with all other groups. Passive LA emptying fraction decreased progressively across the cardiac form stages of Chagas disease. Active LA emptying fraction was increased in patients at stage B compared with controls and stage A patients and was depressed in patients with HF (Table 5).

Except for one patient with the indeterminate form, images of sufficient quality to determine LA ε were obtained from all other patients. LA $\varepsilon_{neg\ peak}$ was significantly depressed only in patients with HF. LA $\varepsilon_{pos\ peak}$ was decreased in stage A patients compared with controls and indeterminate patients and was also lower in patients at stages C and D than in all other groups. LA ε_{tot} was significantly reduced in patients with HF compared with all other groups. Patients at stages A and B also presented lower LA ε_{tot} than indeterminate patients (Table 5, Figure 1).

LV Volume and Function by RT3DE and ε Analysis

LV function was further analyzed using RT3DE and STE to study possible mechanisms related to LV diastolic and LA function. LV

Table 4 LV diastolic functional parameters

Variable	Controls (*n* = 32)	Indeterminate (*n* = 69)	Stage A (*n* = 32)	Stage B (*n* = 25)	Stages C and D (*n* = 26)
E/A ratio	1.4 ± 0.3	1.5 ± 0.4	1.2 ± 0.3*,†	1.4 ± 1.0	1.9 ± 1.3†,‡
DT (msec)	169 ± 33	170 ± 27	186 ± 50	186 ± 66	161 ± 60
AFF (%)	32 ± 6	33 ± 8	36 ± 8	36 ± 11	35 ± 11
IVRT (msec)	93 ± 16	96 ± 19	97 ± 22	108 ± 18*,†	111 ± 36*,†,‡
E′ (cm/sec)	12.6 ± 2.3	12.1 ± 3.1	10.3 ± 2.9*,†	8.3 ± 2.8*,†,‡	5.6 ± 1.9*,†,‡,§
A′ (cm/sec)	9.4 ± 1.8	9.8 ± 2.0	10.5 ± 2.0*	9.0 ± 2.2‡	5.7 ± 3.0*,†,‡,§
E/E′ ratio	4.6 ± 1.7	6.7 ± 1.8*	7.8 ± 2.9*,†	9.6 ± 4.2*,†	17.4 ± 8.5*,†,‡,§
E′/A′ ratio	1.4 ± 0.3	1.3 ± 0.4	1.0 ± 0.3*,†	1.0 ± 0.5*,†	1.2 ± 0.7
S (cm/sec)	48 ± 10	54 ± 12	57 ± 10*	52 ± 16	49 ± 19
D (cm/sec)	49 ± 10	52 ± 11	52 ± 11	49 ± 12	58 ± 21
S/D ratio	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.0 ± 0.5
Ar (cm/sec)	27 ± 4	29 ± 7	32 ± 9*	30 ± 5	30 ± 7
Vp (cm/sec)	75 ± 23	68 ± 24	60 ± 25*	62 ± 29	41 ± 12*,†,‡,§
E/Vp	0.8 ± 0.3	1.2 ± 0.4*	1.4 ± 0.4*	1.5 ± 1.0*	2.2 ± 1.1*,†,‡,§
Untwist (°/sec)	−102 ± 24	−99 ± 36	−97 ± 32	−62 ± 29*,†,‡	−47 ± 25*,†,‡

Data are expressed as mean ± SD.
AFF, Atrial filling fraction; *DT*, E-wave deceleration time; *IVRT*, isovolumic relaxation time.
*$P < .05$ versus controls.
†$P < .05$ versus indeterminate.
‡$P < .05$ versus stage A.
§$P < .05$ versus stage B.

Table 5 Three-dimensional echocardiographic LA volume and function and LA ε analysis

Variable	Controls (*n* = 32)	Indeterminate (*n* = 69)	Stage A (*n* = 32)	Stage B (*n* = 25)	Stages C and D (*n* = 26)
RT3DE					
Maximum LA volume (mL/m^2)	21.0 ± 5.3	21.7 ± 5.8	22.5 ± 5.4	23.6 ± 7.9	39.4 ± 17.6*,†,‡,§
Minimum LA volume (mL/m^2)	7.6 ± 2.4	7.9 ± 2.4	9.2 ± 2.9*,†	10.5 ± 3.8*,†	26.7 ± 17.2*,†,‡,§
Pre-A LA volume (mL/m^2)	10.7 ± 3.1	11.7 ± 3.4	12.9 ± 3.9*	16.0 ± 5.4*,†,‡	32.2 ± 18.6*,†,‡,§
Total LA EF (%)	63 ± 7	63 ± 6	59 ± 8†	55 ± 9*,†	37 ± 18*,†,‡,§
Active LA EF (%)	29 ± 8	32 ± 9	29 ± 10	34 ± 11*,‡	23 ± 12*,†,§
Passive LA EF (%)	48 ± 9	46 ± 9	42 ± 12*	31 ± 11*,†,‡	22 ± 14*,†,‡,§
LA ε					
LA $\varepsilon_{neg\ peak}$ (%)	−12.7 ± 3.2	−12.8 ± 2.5	−13.1 ± 3.1	−12.2 ± 2.8	−7.3 ± 3.0*,†,‡,§
LA $\varepsilon_{pos\ peak}$ (%)	17.2 ± 4.8	17.8 ± 4.6	14.8 ± 4.0*,†	15.2 ± 7.0†	9.3 ± 4.7*,†,‡,§
LA ε_{tot} (%)	30.0 ± 6.2	30.6 ± 5.1	28.0 ± 5.5†	27.4 ± 8.0†	16.6 ± 7.0*,†,‡,§

Data are expressed as mean ± SD.
EF, Emptying fraction.
*$P < .05$ versus controls.
†$P < .05$ versus indeterminate.
‡$P < .05$ versus stage A.
§$P < .05$ versus stage B.

end-diastolic and end-systolic volumes were increased in patients at stage B and further increased in patients with HF. Three-dimensional LV ejection fraction and LV longitudinal, circumferential, and radial ε were decreased in patients at stage B and further decreased in those with HF. Peak LV twist and torsion were decreased in stage A patients compared with controls and further decreased in patients at stage B and those with HF (Table 6).

LA $\varepsilon_{pos\ peak}$ was independently associated with minimum LA volume ($P < .0001$), E/E′ ratio ($P = .006$), E′/A′ ratio ($P < .0001$), and peak LV longitudinal ε ($P < .0001$). LA ε_{tot} was independently associated with maximum LA volume ($P = .02$), E′ velocity ($P = .0008$), and peak LV longitudinal ε ($P < .0001$). LA $\varepsilon_{neg\ peak}$ was independently associated with precontraction LA volume ($P < .0001$), and LV S′ velocity ($P < .0001$).

RT3DE Versus 2D Echocardiography for LA Volume and Function Assessment

Intraclass correlation coefficients between LA volumes determined by RT3DE and 2D echocardiography were 0.76 (95% confidence interval [CI], 0.69–0.81), 0.88 (95% CI, 0.84–0.91), and 0.82 (95% CI, 0.76–0.86) for maximum, minimum, and precontraction LA volumes, respectively. Two-dimensional echocardiography overestimated real-time three-dimensional echocardiographic LA

Table 6 Three-dimensional echocardiographic LV volume and function and LV ε analysis

Variable	Controls	Indeterminate	Stage A	Stage B	Stages C and D
RT3DE	(*n* = 22)	(*n* = 57)	(*n* = 25)	(*n* = 21)	(*n* = 18)
LV end-diastolic volume (mL/m^2)	53 ± 11	54 ± 12	56 ± 10	70 ± 16*,†,‡	100 ± 25*,†,‡,§
LV end-systolic volume (mL/m^2)	21 ± 5	23 ± 7	24 ± 8	39 ± 13*,†,‡	71 ± 30*,†,‡,§
Ejection fraction (%)	59 ± 5	58 ± 7	57 ± 9	45 ± 9*,†,‡	32 ± 16*,†,‡,§
LV ε	(*n* = 28)	(*n* = 67)	(*n* = 30)	(*n* = 23)	(*n* = 26)
Longitudinal (%)	−19 ± 2	−19 ± 2	−19 ± 2	−15 ± 3*,†,‡	−9 ± 4*,†,‡,§
Circumferential (%)	−19 ± 2	−19 ± 4	−19 ± 3	−14 ± 4*,†,‡	−8 ± 4*,†,‡,§
Radial (%)	44 ± 12	46 ± 12	42 ± 13	33 ± 12*,†,‡	17 ± 11*,†,‡,§
Peak apical rotation (°)	9.3 ± 3.7	8.4 ± 4.9	6.7 ± 3.3*	5.4 ± 4.9*,†	3.7 ± 3.5*,†,‡
Peak basal rotation (°)	−5.4 ± 2.6	−4.3 ± 3.6	−4.9 ± 4.0	−3.1 ± 3.2*	−2.4 ± 2.9*,†,‡
Peak twist (°)	13.9 ± 5.1	11.8 ± 6.0	10.9 ± 5.3*	7.7 ± 6.0*,†,‡	5.3 ± 4.4*,†,‡
Peak torsion (°/cm)	1.7 ± 0.6	1.5 ± 0.7	1.4 ± 0.6*	0.8 ± 0.7*,†,‡	0.6 ± 0.5*,†,‡

Data are expressed as mean ± SD.
$^{*}P < .05$ versus controls.
$^{\dagger}P < .05$ versus indeterminate.
$^{\ddagger}P < .05$ versus stage A.
$^{\S}P < .05$ versus stage B.

maximum volume by 23 ± 29%, precontraction LA volume by 36 ± 39%, and LA minimum volume by 14 ± 35%. However, intraclass correlation coefficients between LA volume derived parameters determined by RT3DE and 2D echocardiography ranged from moderate to only slight: 0.57 (95% CI, 0.47–0.66), 0.15 (95% CI, 0.04–0.24), and 0.37 (95% CI, 0.23–0.49) for total, active, and passive LA emptying fractions, respectively.

Survival Analysis

A total of six deaths, 13 admissions for worsening HF, five onsets of atrial fibrillation, four strokes, four admissions for cardiac arrhythmias, and one heart transplantation occurred during a mean follow-up period of 842 ± 245 days. Most of these events (70%) occurred in patients at stage C or D, while 25% of the events occurred in patients at stage B, and only one indeterminate patient had an event. Cox proportional-hazards regression adjusted for age and sex revealed that end-systolic LV diameter (hazard ratio, 1.6; 95% CI, 0.9–2.8); $P = .09$), E′ velocity (hazard ratio, 0.51; 95% CI, 0.34–0.77; $P = .001$), and LA $\varepsilon_{neg\ peak}$ (hazard ratio, 1.21; 95% CI, 1.02–1.44; $P = .03$) were independent predictors of the combined end point.

Intraobserver and Interobserver Variability

The mean differences (± 1.96 SDs) for intraobserver agreement for LA volume on RT3DE were −0.1 ± 3.3 mL/m^2, −0.08 ± 1.51 mL/m^2, and −0.16 ± 1.88 mL/m^2 for maximum, minimum, and precontraction LA volumes, respectively. The mean differences (± 1.96 SDs) for interobserver agreement for LA volume on RT3DE were −0.4 ± 4.3 mL/m^2, −1.0 ± 3.9 mL/m^2, and −1.1 ± 4.7 mL/m^2 for maximum, minimum, and precontraction LA volumes, respectively.

The mean differences (± 1.96 SDs) for intraobserver agreement for LA ε were 0.7 ± 2.9%, −1.0 ± 2.7%, and 1.7 ± 3.9% for global LA $\varepsilon_{pos\ peak}$, LA $\varepsilon_{neg\ peak}$, and LA ε_{tot}, respectively. The mean differences (± 1.96 SDs) for interobserver agreement for LA ε were 3.1 ± 3.8%, −1.3 ± 3.7%, and 4.3 ± 5.3% for global LA $\varepsilon_{pos\ peak}$, LA $\varepsilon_{neg\ peak}$, and LA ε_{tot}, respectively.

DISCUSSION

LV diastolic dysfunction was present in all Chagas disease groups studied and was gradually more prevalent and severe across groups from the chronic indeterminate form to the more advanced stages of the cardiac form. Tissue Doppler, in particular E′ velocity, was the best LV diastolic parameter to identify the progressive worsening of LV diastolic function. LA function was also depressed in patients with Chagas disease. Although LA conductive function was depressed in all groups of patients with the cardiac form, LA contractile function was depressed only in those with HF. Survival analysis revealed that LV diastolic function and LA contractile function were independently associated with the occurrence of clinical events.

LV Diastolic Function

We found a prevalence of LV diastolic dysfunction of 10% in patients with the indeterminate form that increased progressively within patients with the cardiac form from 50% in patients at stage A to 92% in patients with HF. Therefore, diastolic dysfunction predates LV systolic dysfunction in patients with Chagas disease, as shown in other studies,[13,17] and its prevalence increases with the severity of LV systolic dysfunction, as previously shown.[13,14]

Although the prevalence of diastolic dysfunction in patients with the indeterminate form described by us differed from that found in other studies,[17,18] a retrospective study[13] that included a large number of patients with the indeterminate form found a prevalence of delayed relaxation (18%) similar to ours. The use of tissue Doppler allowed us to find a high prevalence of diastolic dysfunction in patients at stage A of the cardiac form, similar to another study that also used tissue Doppler,[17] while another study that did not use tissue Doppler found a lower prevalence.[13] Regarding patients at stage B, our findings concerning tissue Doppler were similar to those of others,[18] and the prevalence of LV diastolic function and severity were similar to those in another study after the exclusion of patients with hypertension.[13] The overwhelming majority of patients with HF in our study presented with LV diastolic patterns associated with high LV filling pressures (pseudonormal and restrictive), similar to the pattern described in a previous work.[13]

Tissue Doppler was the best tool to identify the difference in LV diastolic performance among the studied groups. E′ velocity decreased progressively across the stages of the cardiac form, and E/E′ ratio was increased in all groups compared with controls and in all Chagas heart disease groups compared with patients with the indeterminate form. However, an average E/E′ ratio associated with high LV end-diastolic pressure was found only in patients with HF. The importance of LV diastolic dysfunction in Chagas disease is corroborated by previous studies that described the prognostic value of E/E′ ratio[19] and elevated B-type natriuretic peptide levels in patients with LV diastolic dysfunction without concomitant LV systolic dysfunction.[17,30] Moreover, we found E′ velocity to be independently associated with the occurrence of clinical events.

LA Volume and Function

Our study was the first to analyze LA volume and function in patients with the indeterminate form and at all stages of Chagas heart disease using both RT3DE and STE. Previous studies have evaluated LA function only in patients with Chagas heart disease complicated by HF.[31-33] We also evaluated LA volume and function using 2D echocardiography. However, 2D echocardiography overestimated real-time three-dimensional echocardiographic LA volumes, probably because of geometric assumptions that do not apply to a nonuniform cavity. Moreover, LA volumes measured by RT3DE present a better correlation with LA volumes evaluated by cardiac resonance than 2D echocardiography,[34] and intraclass correlation coefficients between LA volume–derived parameters determined by 2D echocardiography and RT3DE were not satisfactory. Therefore, we preferred to use RT3DE to evaluate LA volume and function in our study.

Maximum LA volume was increased only in patients with HF, while minimum and precontraction LA volumes were already increased in all stages of the cardiac form. These findings may be consequence of the LV diastolic dysfunction found in patients at early stages of the cardiac form. The LV diastolic dysfunction present in all stages of the cardiac form may also contribute to the depression in LA conductive function found in those groups of patients, as demonstrated by the decrease in passive LA emptying fraction and global LA $\varepsilon_{pos\ peak}$ found in those patients. Moreover, LA conductive and reservoir function measured by STE was correlated with LV diastolic and systolic functional parameters.

LA contractile function was depressed only in patients with HF, as active LA emptying fraction and global LA $\varepsilon_{neg\ peak}$ were decreased only in this group of patients, and may contribute to the establishment of HF symptoms. The active LA emptying fraction was increased in patients at stage B, which could have occurred to compensate for the LV diastolic dysfunction present in those patients. Moreover, LA contractile function had prognostic value independent from LV systolic function, which reinforces the importance of the evaluation of LA contractile function.

Clinical Implications

LV diastolic dysfunction was found in patients with the indeterminate form or any of the stages of the cardiac form of Chagas disease. Because E/E′ ratio was described to be a survival predictor in Chagas disease,[19] and E′ velocity was an independent predictor of clinical outcomes in our study, tissue Doppler should always be used to determine LV diastolic function in all patients with Chagas disease, and pulmonary vein flow and LA volumes should be used to further clarify LV diastolic function whenever mitral inflow and tissue Doppler are not enough to clarify patient diagnosis.

Regarding LA volume and function, we found that the correlations between LA function parameters determined by RT3DE and 2D echocardiography were at most moderate. Because LA volumes determined by RT3DE present better correlations with cardiac resonance than 2D echocardiography,[34] RT3DE should be preferred whenever it is necessary to evaluate LA function. Because maximum LA volume is a prognostic marker in Chagas disease,[5] and LA $\varepsilon_{neg\ peak}$ was an independent predictor of clinical events in our study, LA function may emerge as an important component in the evaluation of Chagas disease, and further studies are needed to confirm the value of LA functional analysis in Chagas disease.

Another important finding is the reduced LV twist and torsion we found in patients at stage A of the cardiac form. Those patients characteristically present electrocardiographic changes but no global or segmental LV systolic dysfunction. Therefore, LV torsion analysis was able to identify early changes in LV systolic function in these subjects, and the clinical significance deserves further studies.

Strengths and Limitations

Our study included a large number of patients with the indeterminate form and at all stages of the Chagas heart disease and excluded patients with any associated conditions that could affect diastolic function, and we performed a full evaluation of LV diastolic function using different parameters. We also studied the correlation of LA function with LV diastolic and systolic function. The prospective character of the study allowed the study of the prognostic value of the new echocardiographic parameters compared with traditional ones.

The small number of events and the relatively short follow-up time limited the results of this study. At this time, it is not possible to determine which of these novel echocardiographic parameters are capable of predicting disease progression. Moreover, because most of the reported events occurred in patients at stages B, C, and D of the cardiac form, the predictive value reported in this study for E′ velocity and LA contractile function may not apply to patients with the indeterminate form or at the early stages of the cardiac form. Further studies of the clinical follow-up of the patients included in this study may be useful to elucidate this aspect.

The lack of concomitant analysis of LV diastolic or LA function by invasive hemodynamic assessment and of concomitant analysis of LV or LA volume by cardiac magnetic resonance imaging limit the findings of this study. This study is also limited by the interobserver variability for global LA$\varepsilon_{neg\ peak}$ and LAε_{tot}, although still similar to those previously described.[12]

CONCLUSIONS

LV diastolic dysfunction was found in all forms of chronic Chagas disease, including those without LV systolic dysfunction, but its prevalence and severity gradually increased from the indeterminate form to the more advanced stages of the cardiac form. Tissue Doppler was the best tool to identify the progressive worsening of LV diastolic dysfunction.

Although maximum LA volume was increased only in patients with HF, minimum and precontraction LA volumes were already increased in patients at all stages of the cardiac form of the disease. The changes in LA volume before the development of LV systolic dysfunction point out the importance of our finding of altered diastolic function at early stages of the cardiac form. LA conductive function was depressed at all stages of the cardiac form, while LA contractile function was depressed in patients with HF, as assessed by RT3DE and

STE. The importance of the evaluation of LV diastolic function and LA function in Chagas disease was reinforced by our finding that end-systolic LV diameter, E′ velocity, and LA $\varepsilon_{neg\ peak}$ were independent predictors of clinical events.

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