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Systemic microvascular endothelial dysfunction is associated with left ventricular ejection fraction reduction in chronic Chagas' disease patients.

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ABSTRACT

Objective: This study compares microvascular reactivity (MR) in chronic Chagas' disease (CD) patients with healthy individuals, matched for sex and age. In addition, we evaluated the association between MR and left ventricular ejection fraction (LVEF) in patients. Methods: Acetylcholine iontophoresis was performed on the forearm skin, using laser speckle contrast imaging, to evaluate endothelium-dependent vasodilation. Clinical data were obtained from medical records. Results: Thirty-six patients were compared to 25 healthy individuals (controls). Vasodilation was higher in controls, when compared to patients ($P<0.0001$). There was a significant association between LVEF, stratified into quartiles, and MR (P value for linear trend = 0.002). In addition, there was no difference in MR between patients with normal LVEF and the control group. In patients, MR was independent of the presence of arterial hypertension or diabetes. Conclusions: We have shown for the first time that the reduction of MR is associated with a decrease of LVEF in a cohort of chronic CD patients. The results were not affected by comorbidities, such as hypertension or diabetes. The evaluation of systemic endothelial function may be useful to tailor therapeutic and preventive approaches, targeted at systolic left ventricular failure associated with chronic CD cardiomyopathy.

Keywords: Chagas' disease, microvascular endothelial function, microvasculature, laser speckle contrast imaging, left ventricular ejection fraction.

INTRODUCTION

Chagas' disease (CD) represents the main infectious heart disease in the world, currently affecting 6 million people.[1] Traditionally confined to Central and South America, recent increases of immigration have transformed CD into a global concern.[2] The prognosis of Chagas' cardiomyopathy is worse, when compared with other etiologies.[3] After decades of an asymptomatic stage, the disease can progress to systolic heart failure (HF).[4]

Endothelial dysfunction (ED) is present in a variety of cardiovascular diseases, including the cardiomyopathies.[5] ED is modulated by therapeutic strategies and is associated with worse prognosis, even in asymptomatic individuals.[6]

The skin is an accessible region for the study of the microvasculature, with recent data suggesting the association of cutaneous microvascular ED with other vascular beds, including the coronary circulation.[7] In a previous study from our group,[8] microvascular ED was demonstrated in CD patients with reduced left ventricular ejection fraction (LVEF), when compared to healthy individuals. At the present study, we addressed whether ED is also present in patients with preserved LVEF. These findings could bring insight to cardiovascular pathophysiology of CD. In addition, microcirculation assessment could be suggested as a possible target for therapeutic interventions and with a possible role in the clinical evaluation of CD patients.

METHODS

Study Design and Patients

This was a cross-sectional study of 36 consecutive adult patients treated at a CD outpatient clinic in a quaternary hospital. Eligibility criteria were age ≥ 18 years, with two serologies (with different methods) positive for CD. The exclusion criterion was decompensated HF (New York Heart Association functional class IV or impairment over the previous month). We also recruited 25 healthy control subjects, matched for age and sex, without history of both cardiovascular and metabolic diseases. The study was approved by the local Institutional Review Board (IRB) under protocol # CAAE 47563415.9.0000.5272, and informed consent was obtained from all participants; the study protocol was registered on clinicaltrials.gov with the number NCT03524768.

Anthropometry (height, body weight, and body mass index), comorbidities, smoking history, plasma biochemistry, medications, electrocardiographic and echocardiographic parameters were obtained from medical records. Data were analyzed when obtained in the previous 12 months before recruitment.

Evaluation of skin microvascular reactivity

Microvascular reactivity was evaluated as previously described,[9] using a laser speckle contrast imaging (LSCI) system with a laser wavelength of 785 nm (PeriCam PSI system, Perimed, Järfälla, Sweden). The measurements were expressed in arbitrary perfusion units (APU). The images were analyzed using PIM Soft software (Perimed, Järfälla, Sweden). Acetylcholine (Ach) 2% w/v (Sigma Chemical CO, St. Louis, USA) iontophoresis was performed using a micropharmacology system (PF 751 Perilont USB Power Supply, Perimed, Sweden) with increasing anodal currents of 30, 60, 90, 120, 150 and 180 μA, administered for 10-second intervals. Skin blood flow measurements, expressed in APU, were divided by the mean arterial pressure to yield the cutaneous vascular conductance (CVC) in APU/mmHg. Results were expressed as area under the curve (AUC) of Ach-induced vasodilation.

Statistical analysis

Variables were tested for normality using the Shapiro-Wilk normality test. Comparisons of numerical variables with normal distribution were analyzed by Student-t test, and variables with non-Gaussian distribution were analyzed by Mann-Whitney test. Categorical variables were analyzed by Chi-Square or Fisher's exact test. The effects of increasing currents of Ach iontophoresis are shown as the mean (95% confidence interval). Statistical analyses were performed using two-way ANOVA followed by multiple comparisons (Sidak's multiple comparisons test), where we considered the interactions of Ach dose and groups of individuals (patients and healthy volunteers). P values < 0.05 were considered significant. The results of normal distribution variables were presented as mean ± SD. Non-Gaussian variables were presented as median and interquartile ranges. Statistical analyses were performed using software Prism version 6.0 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Thirty-six patients were recruited between April and October 2018. Anthropometric and laboratory characteristics of CD patients and the control group are presented in Table 1. Cardiovascular medications used by patients are presented in Table 2. There were no current smokers in either group. The main comorbidities in the patient group were hypertension (n=29, 80.5%), dyslipidemia (n=26, 72.2%), and diabetes (n=10, 27.8%). There were 25 healthy individuals in the control group, with no differences compared to patients regarding age, sex, and body mass index. The endothelial-dependent effects of microcurrents superior to 120 μA of Ach iontophoresis were significantly higher in healthy individuals, compared with CD patients (Figure 1). The maximum increase of CVC from baseline was of 106% (95% CI of mean 81-130) and 48% (95% CI of mean 32-64), respectively, P<0.0001. The values of AUC of Ach-induced vasodilation in patients were stratified into quartiles of LVEF (n=9 in each group): quartile 1 (≤40.6%), quartile 2 (>40.6 and ≤60.2%), quartile 3 (>60.2 and ≤71.6%), and quartile 4 (>71.6%). AUC in each quartile was respectively of 15,456, 12,482, 20,656 and 27,302 APU/s. There was a significant association between LVEF and endothelial-dependent microvascular reactivity in CD patients (P-values for linear trend = 0.002) (Figure 2 A). In addition, there was no difference in vasodilation between patients with LVEF >60.2% [n=18; AUC values of 20,796 (16,770 – 27,445) APU/s] and the healthy group [n=25; AUC values of 21,049 (14,277 – 22,986) APU/s; P=0.48, Figure 2 B]. In order to evaluate the possible contribution of hypertension and diabetes mellitus to endothelial function in CD patients, we compared individuals with or without these two conditions, finding no difference in the response to Ach (Table 3).

DISCUSSION

Microvascular ED has been recognized in systemic vessels of patients presenting advanced HF with reduced LVEF of either ischemic or idiopathic etiologies,[10, 11] and previously by our group in CD patients.[8] An issue of interest was to gain insight into the moment of ED observation in the course of CD. Multiple lines of evidence link the pathophysiology of ED in HF to the mechanisms underlying systolic dysfunction.

Inflammation is a central element of Chagas cardiomyopathy.[12] Patients in the chronic phase of CD have increased plasma levels of cytokines, primarily INF-γ, IL-6 and TNF-α [13-15]. The latter is considered one of the most potent inhibitory stimuli for endothelial nitric oxide synthase (eNOS) expression in vascular endothelial cells.[16] In addition, cyclic GMP, the downstream mediator of nitric oxide promoting vascular smooth muscle relaxation, attenuates both ventricular remodeling and systolic dysfunction.[17] IL-6 induces vascular oxidative stress mediated by the key enzyme nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase,[18] which synthesizes hydrogen peroxide, inactivating NO and converting it to another oxidant which does not induce vasodilation, peroxynitrite. The decrease of cardiac output resulting from left ventricular systolic dysfunction may be deleterious for microvascular function *per se*, through two mechanisms. First, it is associated with chronically reduced endothelial shear stress, which down-regulates eNOS expression.[19] Second, chronically low cardiac output may induce a mismatch between oxygen supply and consumption, favoring a state of hypoxia in peripheral tissues.[20] Moreover, recent evidence obtained from cultured human endothelial cells suggests that hypoxia stimulates vascular oxidative stress with mitochondrial production of superoxide.[21]

Another mechanism proposed as related to the progression of CD cardiomyopathy and observed in patients with preserved LVEF is vascular dysautonomy,[22] which is also associated with ED in chronic CD patients.[23]

In order to evaluate the degree of microvascular ED in chronic CD patients, we compared the patient group with healthy individuals, matched for sex, age, and body mass index. To the best of our knowledge, the current study is the largest comparing microcirculation in chronic CD patients with healthy counterparts, and the first comprising a spectrum of patients displaying different degrees of left ventricular function.

Hypertension and diabetes are extensively recognized as conditions associated with ED.[24, 25] We found no difference between CD patients with and without these comorbidities, suggesting that the endothelial-dependent vasodilation results observed are related to CD *per se*.

Chronic chest pain is a common complaint in CD patients.[26] Coronary microvascular ED has been observed in CD animal models[27] and patients.[28] Accordingly, coronary microcirculatory dysfunction may play a role in the progression of chronic CD, inducing recurrent episodes of ischemia, which could contribute to HF development.[29] Studies comparing simultaneously cardiac and cutaneous microcirculation in the same group of patients could further clarify whether systemic ED advances in conjunction with left ventricular failure in CD.

Although microvascular ED has been previously proposed as a risk factor for adverse cardiovascular outcomes, including angina and stroke,[6] our present findings are hypothesis generating, and do not support the prognostic use of cutaneous microvascular evaluation in CD patients. Indeed, in the present study ED was absent in patients with preserved LVEF, even though most of them displayed electrocardiogram and echocardiogram abnormalities typical of CD according to Guidelines,[30, 31] like regional wall motion deficits (supplemental Figure 1). Our current findings, at the microcirculation level, support previous data, which found no ED in the brachial artery of chronic CD patients with preserved LVEF.[32] Follow-up studies in patients with preserved LVEF may further ascertain the temporal correspondence between endothelial and ventricular dysfunction in the chronic phase of CD.

LIMITATIONS OF THE STUDY

The present study was undertaken in a single healthcare center with 36 patients, and therefore may not be representative of a large population of CD patients. Medications could not be interrupted for ethical reasons, and may interfere with the vascular reactivity results. In addition, as a cross-sectional study, this investigation cannot address a cause-and-effect relationship between ED and LVEF reduction. Our present findings suggest that MR deteriorates in parallel with LVEF. Whether these are epiphenomena related to the same mechanism (i. e. inflammation) or MR dysfunction precedes (or even follows) LVEF decrease, is beyond the scope of the present study. Microcirculation reactivity was assessed using skin iontophoresis for non-invasive transdermal drug delivery, which is based on the transfer of charged molecules using a low-intensity electric current.[33] In this method, the

intensity and the duration of the current, as well as the nature of the skin surface (thickness, glabrous or not), may play a crucial role. Nevertheless, it is noteworthy that Ach iontophoresis has been standardized[9] and extensively used by our research group to assess microvascular endothelium-dependent vasodilation in cardiovascular and metabolic diseases, including arterial hypertension[34], and diabetes.[35]

PERSPECTIVES:

• Systemic microvascular reactivity (SMR) is impaired in chronic Chagas disease (CD) patients
• SMR dysfunction is associated with left ventricular ejection fraction reduction
• Microvascular endothelial function may be useful to tailor therapeutic approaches in CD patients

CONCLUSIONS

Our data suggest, for the first time, that the decline of systemic endothelial microvascular reactivity is associated with the degree of LVEF reduction in chronic CD patients. Patients with preserved LVEF had similar endothelium-dependent microvascular reactivity when compared to healthy volunteers. The results were not influenced by coexisting arterial hypertension or diabetes. Systemic microvascular endothelial function measured at the cutaneous circulation may be a useful non-invasive tool in the clinical assessment of patients with the cardiac form of CD.

DECLARATIONS:

Ethics approval and consent to participate:

The present study was conducted in accordance with the Declaration of Helsinki, and this study was approved by the Institutional Review Board of the National Institute of Cardiology in Rio de Janeiro, Brazil, under protocol # 47563415.9.0000.5272. Written informed consent to participate in the study was obtained from all participants.

Author contributions: DK and ET contributed to the conception and design of the study, and to the analysis and interpretation of data; DK, AB, AL, VV and ET were involved in the drafting of the manuscript, and literature review. All authors have given final approval of the version to be published and are publicly responsible for its content.

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Competing interests

The authors declare that they have no competing interests.

Data availability statement: The data that support the findings of this study are available from the corresponding author, ET, upon reasonable request.

REFERENCES

1. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. *Wkly Epidemiol Rec* 2015;90:33-43.

2. Gascon J, Bern C, Pinazo MJ. Chagas disease in Spain, the United States and other non-endemic countries. *Acta Trop* 2010;115:22-27.

3. Traina MI, Sanchez DR, Hernandez S, Bradfield JS, Labedi MR, Ngab TA, Steurer F, Montgomery SP, Meymandi SK. Prevalence and Impact of Chagas Disease Among Latin American Immigrants With Nonischemic Cardiomyopathy in Los Angeles, California. *Circ Heart Fail* 2015;8:938-943.

4. Nunes MCP, Beaton A, Acquatella H, Bern C, Bolger AF, Echeverria LE, Dutra WO, Gascon J, Morillo CA, Oliveira-Filho J, Ribeiro ALP, Marin-Neto JA. Chagas Cardiomyopathy: An Update of Current Clinical Knowledge and Management: A Scientific Statement From the American Heart Association. *Circulation* 2018;138:e169-e209.

5. Bauersachs J, Widder JD. Endothelial dysfunction in heart failure. *Pharmacol Rep* 2008;60:119-126.

6. Anderson TJ, Charbonneau F, Title LM, Buithieu J, Rose MS, Conradson H, Hildebrand K, Fung M, Verma S, Lonn EM. Microvascular function predicts cardiovascular events in primary prevention: long-term results from the Firefighters and Their Endothelium (FATE) study. *Circulation* 2011;123:163-169.

7. Tibirica E, Souza EG, De Lorenzo A, Oliveira GM. Reduced systemic microvascular density and reactivity in individuals with early onset coronary artery disease. *Microvasc Res* 2014;97:105-108.

8. Borges JP, Mendes F, Lopes GO, Sousa AS, Mediano MFF, Tibirica E. Is endothelial microvascular function equally impaired among patients with chronic Chagas and ischemic cardiomyopathy? *Int J Cardiol* 2018;265:35-37.

9. Cordovil I, Huguenin G, Rosa G, Bello A, Kohler O, de Moraes R, Tibirica E. Evaluation of systemic microvascular endothelial function using laser speckle contrast imaging. *Microvasc Res* 2012;83:376-379.

10. Fischer D, Rossa S, Landmesser U, Spiekermann S, Engberding N, Hornig B, Drexler H. Endothelial dysfunction in patients with chronic heart failure is independently associated with increased incidence of hospitalization, cardiac transplantation, or death. *Eur Heart J* 2005;26:65-69.

11. Witman MA, Fjeldstad AS, McDaniel J, Ives SJ, Zhao J, Barrett-O'Keefe Z, Nativi JN, Stehlik J, Wray DW, Richardson RS. Vascular function and the role of oxidative stress in heart failure, heart transplant, and beyond. *Hypertension* 2012;60:659-668.

12. Cunha-Neto E, Chevillard C. Chagas disease cardiomyopathy: immunopathology and genetics. *Mediators Inflamm* 2014;2014:683230.

13. Abel LC, Rizzo LV, Ianni B, Albuquerque F, Bacal F, Carrara D, Bocchi EA, Teixeira HC, Mady C, Kalil J, Cunha-Neto E. Chronic Chagas' disease cardiomyopathy patients display an increased IFN-gamma response to Trypanosoma cruzi infection. *J Autoimmun* 2001;17:99-107.

14. Poveda C, Fresno M, Girones N, Martins-Filho OA, Ramirez JD, Santi-Rocca J, Marin-Neto JA, Morillo CA, Rosas F, Guhl F. Cytokine profiling in Chagas disease: towards understanding the association with infecting Trypanosoma cruzi discrete typing units (a BENEFIT TRIAL sub-study). *PLoS One* 2014;9:e91154.

15. Ferreira RC, Ianni BM, Abel LC, Buck P, Mady C, Kalil J, Cunha-Neto E. Increased plasma levels of tumor necrosis factor-alpha in asymptomatic/"indeterminate" and Chagas disease cardiomyopathy patients. *Mem Inst Oswaldo Cruz* 2003;98:407-411.

16. Yan G, You B, Chen SP, Liao JK, Sun J. Tumor necrosis factor-alpha downregulates endothelial nitric oxide synthase mRNA stability via translation elongation factor 1-alpha 1. *Circ Res* 2008;103:591-597.

17. Hammond J, Balligand JL. Nitric oxide synthase and cyclic GMP signaling in cardiac myocytes: from contractility to remodeling. *J Mol Cell Cardiol* 2012;52:330-340.

18. Didion SP. Cellular and Oxidative Mechanisms Associated with Interleukin-6 Signaling in the Vasculature. *Int J Mol Sci* 2017;18:

19. Giannitsi S, Bougiakli M, Bechlioulis A, Naka K. Endothelial dysfunction and heart failure: A review of the existing bibliography with emphasis on flow mediated dilation. *JRSM Cardiovasc Dis* 2019;8:2048004019843047.

20. De Boer RA, Pinto YM, Van Veldhuisen DJ. The imbalance between oxygen demand and supply as a potential mechanism in the pathophysiology of heart failure: the role of microvascular growth and abnormalities. *Microcirculation* 2003;10:113-126.

21. Liang X, Arullampalam P, Yang Z, Ming XF. Hypoxia Enhances Endothelial Intercellular Adhesion Molecule 1 Protein Level Through Upregulation of Arginase Type II and Mitochondrial Oxidative Stress. *Front Physiol* 2019;10:1003.

22. Rabelo DR, Rocha MO, de Barros MV, Silva JL, Tan TC, Nunes MC. Impaired coronary flow reserve in patients with indeterminate form of Chagas' disease. *Echocardiography* 2014;31:67-73.

23. Truccolo AB, Dipp T, Eibel B, Ribeiro RA, Casali KR, Irigoyen MC, Gus I, Pellanda LC, Plentz RD. Association between endothelial function and autonomic modulation in patients with Chagas disease. *Arq Bras Cardiol* 2013;100:135-140.

24. Dharmashankar K, Widlansky ME. Vascular endothelial function and hypertension: insights and directions. *Curr Hypertens Rep* 2010;12:448-455.

25. Avogaro A, Albiero M, Menegazzo L, de Kreutzenberg S, Fadini GP. Endothelial dysfunction in diabetes: the role of reparatory mechanisms. *Diabetes Care* 2011;34 Suppl 2:S285-290.

26. Simoes MV, Ayres EM, Santos JL, Schmidt A, Pintya AO, Maciel BC, Marin-Neto JA. [Detection of myocardial ischemia in chronic Chagas disease patients with atypic precordial pain by exercise and Holter tests]. *Arq Bras Cardiol* 1993;60:315-319.

27. Andrade ZA, Andrade SG, Sadigursky M, Wenthold RJ, Jr., Hilbert SL, Ferrans VJ. The indeterminate phase of Chagas' disease: ultrastructural characterization of cardiac changes in the canine model. *Am J Trop Med Hyg* 1997;57:328-336.

28. Marin-Neto JA, Marzullo P, Marcassa C, Gallo Junior L, Maciel BC, Bellina CR, L'Abbate A. Myocardial perfusion abnormalities in chronic Chagas' disease as detected by thallium-201 scintigraphy. *Am J Cardiol* 1992;69:780-784.

29. Rossi MA, Tanowitz HB, Malvestio LM, Celes MR, Campos EC, Blefari V, Prado CM. Coronary microvascular disease in chronic Chagas cardiomyopathy including an overview on history, pathology, and other proposed pathogenic mechanisms. *PLoS Negl Trop Dis* 2010;4:

30. Andrade JP, Marin-Neto JA, Paola AA, Vilas-Boas F, Oliveira GM, Bacal F, Bocchi EA, Almeida DR, Fragata Filho AA, Moreira Mda C, Xavier SS, Oliveira Junior WA, Dias JC. [I Latin American guidelines for the diagnosis and treatment of Chagas cardiomyopathy]. *Arq Bras Cardiol* 2011;97:1-48.

31. Dias JC, Ramos AN, Jr., Gontijo ED, Luquetti A, Shikanai-Yasuda MA, Coura JR, Torres RM, Melo JR, Almeida EA, Oliveira W, Jr., Silveira AC, Rezende JM, Pinto FS, Ferreira AW, Rassi A, Fragata AAF, Sousa AS, Correia DF, Jansen AM, Andrade GM, Britto CF, Pinto AY, Rassi A, Jr., Campos DE, Abad-Franch F, Santos SE, Chiari E, Hasslocher-Moreno AM, Moreira EF, Marques DS, Silva EL, Marin-Neto JA, Galvao LM, Xavier SS, Valente SA, Carvalho NB, Cardoso AV, Silva RA, Costa VM, Vivaldini SM, Oliveira SM, Valente VD, Lima MM, Alves RV. [Brazilian Consensus on Chagas Disease, 2015]. *Epidemiol Serv Saude* 2016;25:7-86.

32. Consolim-Colombo FM, Lopes HF, Rosetto EA, Rubira MC, Barreto-Filho JA, Baruzzi AC, Rocha NN, Mady C, Irigoyen MC, Krieger EM. Endothelial function is preserved in Chagas' heart disease patients without heart failure. *Endothelium* 2004;11:241-246.

33. Roustit M, Cracowski JL. Non-invasive assessment of skin microvascular function in humans: an insight into methods. *Microcirculation* 2012;19:47-64.

34. Verri V, Brandao AA, Tibirica E. Penile microvascular endothelial function in hypertensive patients: effects of acute type 5 phosphodiesterase inhibition. *Braz J Med Biol Res* 2018;51:e6601.

35. Gomes MB, Matheus AS, Tibirica E. Evaluation of microvascular endothelial function in patients with type 1 diabetes using laser-Doppler perfusion monitoring: which method to choose? *Microvasc Res* 2008;76:132-133.

Table 1: Clinical characteristics of patients with chronic Chagas disease and sex- and age-matched healthy individuals.

Parameter	Chagas (n=36)	Healthy (n=25)	P-Value
Age (years)	67.1 ± 9.9	65.2 ± 5.5	0.37
Male n (%)	11 (29.7)	12 (48)	0.18
Body mass index (kg/m²)	26.2 ± 4.7	26.2 ± 5.1	0.97
Fasting glucose (mg/dL)	104.3±19,2	98.6±6.6	0.18
Total cholesterol (mg/dL)	183.9±37.5	211.4±25.1	0.004
MAP (mmHg)	104.8±14.1	96.2±8.8	0.009
NYHA class (I:II)	21:15	—	—
Electrocardiogram			
Sinus rhythm/AF/PM	27/3/6	25/0/0	
Echocardiogram			
LVEF (%, Teicholz)	58.0 ± 17.1	—	—
EDV (ml)	137.4±50.2		
PASP (mmHg)	31.3±8.6		

The results are presented as the mean ± SD or absolute numbers and percentages. Comparisons of numerical variables were analyzed by Student-t test, and categorical variables were analyzed by Chi-Square or Fisher's exact test.

Abbreviations: AF, atrial fibrillation; EDV, end-diastolic volume; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; PM, pacemaker.

Table 2: Cardiovascular medication use in chronic Chagas disease patients (n=36).

Medication	Number of patients (%)
Angiotensin-converting enzyme inhibitors	6 (16.7)
Angiotensin II receptor antagonists	24 (66.7)
Aldosterone antagonists	8 (22.2)
Diuretics	22 (61.1)
β-blockers	22 (61.1)
Coumarin derivatives / NOACS	1 / 2 (2.8 / 5.6)
Antiplatelet drugs	9 (25)
Statin	22 (61.1)
Hydralazine	9 (25)
Methyldopa	5 (13.9)
Nitrates	2 (5.6)
Calcium channel blockers	5 (13.9)

Abbreviations; NOACS, novel oral anticoagulants.

Table 3 - Assessment of the effects of cutaneous iontophoresis of acetylcholine (Ach) on cutaneous microvascular conductance (CVC), expressed in arbitrary perfusion units (APU, per mean arterial pressure in mmHg), in subgroups of patients with chronic Chagas disease associated with arterial hypertension or diabetes.

Parameters	**HYPERTENSIVE PATIENTS (n=29)**	**NORMOTENSIVE PATIENTS (n=7)**	**P-values**
BASELINE CVC (APU/mmHg)	0.32 ± 0.10	0.31 (0.25-0.32)	0.83
AUC Ach (APU/sec)	19,570 ± 6,359	18,479 ± 7,211	0.69

Parameters	**DIABETIC PATIENTS (n=10)**	**NONDIABETIC PATIENTS (n=26)**	**P-values**
BASELINE CVC (APU/mmHg)	0.28 ± 0.12	0.32 (0.26-0.36)	0.26
AUC Ach (APU/sec)	19,124 ± 7,722	19,457± 6,069	0.89

The results are shown as the mean ± standard deviation or median (25^{th} - 75^{th} percentile), for parametric and nonparametric values, respectively (Shapiro-Wilk normality test). Statistical analyses were performed using two-tailed unpaired *t* tests or two-tailed Mann Whitney tests for parametric and nonparametric values, respectively.

Abbreviations: Ach, Acetylcholine; APU. arbitrary perfusion units (APU); AUC, area under the curve of vasodilation induced by Ach; CVC, cutaneous vascular conductance.

FIGURE LEGENDS

FIGURE 1: Effects of cutaneous iontophoresis of acetylcholine (Ach) on cutaneous vascular conductance, expressed in percentage increases from baseline values, in patients with chronic Chagas disease (CHAGAS) and sex- and age-matched healthy individuals (HEALTHY).

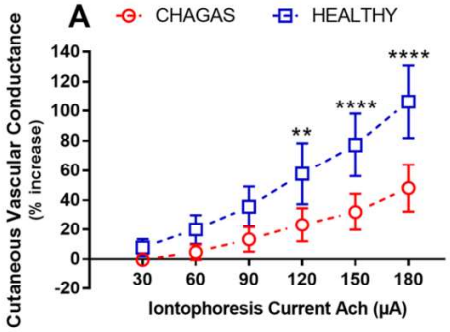
The results are shown as the mean (95% confidence interval). Statistical analyses were performed using two-way ANOVA followed by multiple comparisons (Sidak's multiple comparisons test), where we considered the interactions of Ach dose and groups of individuals (patients and healthy volunteers).

**P<0.01 and ****P<0.0001 vs. patients with chronic Chagas disease.

FIGURE 2: (A) Linear trend of the area under the curve (AUC) of microvascular vasodilation resulting from the cutaneous iontophoresis of acetylcholine (Ach), expressed in quartiles of left ventricular ejection fraction in patients with chronic Chagas disease. (B) Comparison of AUC between Chagas patients with preserved left ventricular ejection fraction and age and sex-matched healthy individuals.

The results are shown as box & whisker plots showing mean, interquartile range and extreme values. Statistical analyses were performed using one-way ANOVA followed by posttest for linear trend (A) or two-tailed unpaired *t*-test (B).

Supplemental Figure 1: Echocardiographic and electrocardiographic abnormalities observed in patients with preserved LVEF. ECHO; echocardiogram. EKG; electrocardiogram.



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